



HANDBOUND  
AT THE



UNIVERSITY OF  
TORONTO PRESS





Digitized by the Internet Archive  
in 2009 with funding from  
University of Toronto







4

4

# *The Journal*

of

## *Laboratory and Clinical Medicine*

VICTOR C. VAUGHAN, M.D., Editor-in-Chief  
University of Michigan, Ann Arbor

### ASSOCIATE EDITORS

#### *Pharmacology*

DENNIS E. JACKSON, M.D.  
University of Cincinnati, Cincinnati

#### *Immunology and Serology*

FREDERICK P. GAY, M.D.  
University of California, Berkeley

#### *Bacteriology*

HANS ZINSSER, M.D.  
Columbia University, New York

#### *Physiological Pathology*

PAUL C. WOOLLEY, M.D.  
University of Cincinnati, Cincinnati

#### *Physiological Chemistry and Clinical Physiology*

J. J. R. MACLEOD, M.B.  
University of Toronto, Toronto

ROY G. PEARCE, M.D.  
Lakeside Hospital, Cleveland

#### *Clinical Microscopy and Laboratory Technic*

ROGER S. MORRIS, M.D.  
University of Cincinnati, Cincinnati

#### *Neuropathology*

E. E. SOUTHWARD, M.D.  
Harvard University, Boston

#### *Tuberculosis*

GERALD B. WEBB, M.D.  
Cragmor Sanatorium, Colorado Springs

148645

21 / 2 / 19

VOLUME III  
OCTOBER, 1917—SEPTEMBER, 1918

ST. LOUIS  
THE C. V. MOSBY CO.  
1918



R

850

J66

v. 3

cop. 2

# *The Journal of Laboratory and Clinical Medicine*

---

VOL. III.

ST. LOUIS, OCTOBER, 1917

No. 1.

---

## ORIGINAL ARTICLES

---

### THE BEARING OF ANTITYPHOID VACCINATION ON THE DIAGNOSTIC VALUE OF THE AGGLUTINATION TEST IN TYPHOID AND PARATYPHOID FEVER

---

BY MAJOR E. RIST, FRENCH ARMY MEDICAL CORPS.

---

THAT the inoculation of typhoid vaccine causes specific agglutinating properties to appear in the blood of the normal individual is a very well known fact. It is even assumed that the degree and duration of those properties can, to a certain extent, be relied upon to ascertain whether the vaccinated individual is really immune, or, having been immune, continues to be so. To many medical men, however, such an assumption will appear too far reaching. Even in the earlier days of his discovery, Widal had repeatedly shown that agglutinating properties are not immunity reactions and must be carefully distinguished from them. Moreover, the serum of an individual who has previously had genuine typhoid, does not, as a rule, exhibit any agglutinative properties after a year or two have elapsed, unless he happens to remain a permanent carrier; and yet the strongest and most durable power of immunity is the one procured by a previous attack of the disease. Nevertheless, agglutination certainly indicates that inoculation has taken place and has provoked some kind of body reaction; and, as we have no better means of ascertaining it, the complement-fixation test being, as far as this problem is concerned, no more reliable, we have to make use of it, although with due regard to its only relative value.

Now the fact that the serum of a previously vaccinated individual agglutinates *B. typhosa* must evidently impair the diagnostic significance of the Widal method, and the question arises: if a patient who has been previously vaccinated exhibits clinical symptoms pointing to the possibility of typhoid, are we in a position to make an accurate diagnosis by using the agglutination test?

If he does not agglutinate, is typhoid to be excluded? If he does, have we any means to prove whether the positive Widal is due to actual infection or to his having been vaccinated? It has been generally assumed that the study of the agglutination titer enables us to do this. In consequence, at least two consecutive quantitative tests should be made at an interval of some days. Many elaborate devices have been proposed in order to facilitate the quantitative estimation of the agglutinative power and to render it more accurate. The Dreyer-Walker method to this effect has even been standardized in the British A. M. C. and has been extensively used in the British Army. Unfortunately when it comes to appreciating the behavior of the agglutination titer and to define the characteristics which belong to the vaccinated-infected type as distinguished from the vaccinated-noninfected, there is no consensus of opinion. Some workers give the foremost importance to chronological considerations, namely to the earlier or later appearance of the agglutinative property or to the rapidity of increase of its titer; others concern themselves with the absolute value of the titer. All, more or less reluctantly, agree to say that there are difficult cases and even cases when the diagnosis based solely upon agglutination is impossible. But they regard those cases as exception, which, on the whole, do not impair the value of the agglutination test.

This is not the only problem raised by antityphoid vaccination. We have to consider also whether previous vaccination against *B. typhosus* affects, and how it affects, the behavior of agglutination of *B. paratyphosus* A or B in individuals infected with one of these germs. In other words, does the test enable us to make a correct diagnosis of paratyphoid A or B in a patient who has been previously vaccinated against *B. typhosus*? This question has also been generally answered in a positive way. One admits that, whether the individual has been previously vaccinated or not, his agglutination titer will be higher with the germ responsible for his actual infection than with *B. typhosus*. On this matter also, it is generally conceded, however, that there are difficult cases, that sometimes even one may be misled by the agglutination test. Caution is therefore recommended. But on what principles this caution should rest is never clearly stated.

In the rather abundant literature relating to these problems, I have not been able to find out the ground for the assumptions made that (1) there is a specific difference in the behavior of the agglutination titer in vaccinated and nonvaccinated individuals and that (2) the diagnostic value of agglutination of *b. paratyphoid* A or B remains practically unimpaired by previous antityphoid vaccination. There are, as a matter of fact, *a priori* assumptions, which indeed seem pretty logical and natural at first view, but which nevertheless must be carefully controlled and tested before they can be admitted. The assumption that the difficult and misleading cases are exceptional does not rest on a sounder basis, the only sound basis in the matter being a numerical one.

The percentage of doubtful and mistaken diagnoses—controlled by blood culture—must be ascertained accurately before we can appreciate correctly the practical value of the agglutination test. Only a very low percentage would leave it unimpaired for practical purposes.

I must confess that when I was entrusted, early in 1915, with the typhoid

department of the French military hospital No. 15, in the 6th Army, I myself shared most of the *a priori* assumptions which have just been alluded to. But I determined to control them as extensively as possible, and I propose to give here a short account of my control researches.<sup>1</sup>

During the whole period of my typhoid work (February, 1915-July, 1916) the French Army was being vaccinated against *B. typhosus* only, the vaccine used being of the Vincent type (culture killed by ether) and four injections at a week's interval being required for a complete vaccination. The inoculations with triple vaccine (*B. typhosus* and BB. paratyphosus A and B) have been initiated much later.

Blood culture was the routine method of diagnosis in my department, being performed in each separate case, and giving an average of 75 p. 100 positive results. The organisms cultivated from the blood were differentiated on the various sugars and on neutralized, doubtful strains being further tested with high agglutinating specific sera prepared by the Pasteur Institute.

Whether the blood culture was positive or not, the serum of each patient was tested for its agglutinating power with every one of the three germs, *B. typhosus*, *B. paratyphosus* A, and *B. paratyphosus* B. This threefold quantitative test was repeated every second day during the whole course of the disease and, in many cases, during convalescence. The quantitative rates of agglutination were plotted on the temperature chart, enabling us to have a clear view of the behavior of both agglutination and coagglutination in each case.

The method used was the microscopic one, with a twenty-four hour old broth culture of a standard not spontaneously agglutinating germ. The dilution began with 1:50 and went up to 1:100, 1:200, 1:300 and so on until a dilution was found with which no clots of bacilli could be seen. If, for instance, clots were still distinguishable at 1:800 but disappeared at 1:900, the agglutination titer was plotted at 800.

My aim was: (1) to make a careful study of the agglutination curves in all cases where the blood culture was positive, in order to see if a clue could be obtained for interpreting diagnostically the same curves in patients having a repeatedly negative blood culture but exhibiting clinical evidence of typhoid, (2) to study furthermore the behavior of agglutination in a number of evidently nontyphoid patients who had been previously vaccinated against *B. typhosus*.

The total number of quantitative tests thus performed from February to November, 1915, amounted to 11,648.

#### I. NONTYPHOID CASES.

Out of 128 positively nontyphoid cases, previously vaccinated, 94 had a positive Widal (76.5 p. 100).

<sup>1</sup>For more detailed statement and tabulation of all the individual cases of my statistics, see: E. Rist, *Étu des sur Fievre Typhoide*, I, *Annales de Medecine*, iii, 54-87, January, 1916.

TABLE I.

	No. of cases	Out of which agglutinated
Angina, acute pharyngitis	8	7
Hypertrophic rhinitis, coryza	15	13
Atrophic rhinitis	1	1
Acute laryngitis	3	2
Maxillary sinusitis	1	1
Pneumonia	13	9
Pulmonary tuberculosis	3	2
Peritoneal tuberculosis	2	1
Meningeal tuberculosis	1	0
Pleural effusion (tuberc.)	4	3
Pleuritis (without effusion)	1	1
Angor pectoris	1	0
Mitral stenosis	1	1
Acute rheumatic fever with valvular involument	4	4
Erythema nodosum	1	1
Lumbago	1	1
Constipation	9	7
Simple diarrhea	12	10
Dysenteric diarrhea	5	4
Amoebic dysentery	2	1
Acute appendicitis	6	6
Duodenal ulcer	1	1
Colitis	1	1
Dyspepsia	1	1
Hernia	1	0
Hemorrhagic jaundice	4	2
Gall stones	1	0
Malaria	11	4
Ephemeral fever of undetermined origin	3	2
Malta fever	2	1
Trigeminal neuralgia	1	0
Shell shock	2	1
Fatigue	6	6

In a majority of cases, *B. typhosus* alone was agglutinated; but there was also a certain amount of coagglutination; and, in a few instances, one of the paratyphoid germs was agglutinated alone:

TABLE II.

b. typhosus alone	70 cases
b. typh. and para. A plus para. B	2
b. typh. plus para. A	13
b. typh. plus para. B	5
b. para. A alone	2
b. para. B alone	2

The maximum agglutination titer for *B. typhosus* has varied from 50 to 1500. I have tabulated the percentage of titer in those nontyphoid patients to compare with the same percentage among my nonvaccinated and my vaccinated typhoid patients (See Table III).

The low titers are more frequent in the first group, the high titers in the second and third groups. But the difference is evidently not striking enough to have the slightest diagnostical value. Nor has the titer remained constant



TABLE III.

Agglutination titer	Nontyphoid (90) (vaccinated)	Typhoid (19) nonvaccinated	Typhoid (27) vaccinated
50	18.8%	0	3.7%
100	16.6	10.5	3.7
200	21.1	5.2	11.1
300	13.3	10.5	18.5
400	10	21	0
500	8.8	15.7	3.7
600	3.3	5.2	11.1
700	1.1	0	11.1
800	2.2	5.2	0
900 and over	4.4	26.3	37.7

during the course of observation in every nontyphoid case. One patient, for instance, was admitted with rheumatic fever and valvular endocarditis, temperature  $38.8^{\circ}$  C. on admission. He was put on sodium salicylate; his temperature went down to  $36.6^{\circ}$  on the fifth day after admission and continued normal, the pain and swelling of the joints disappearing rapidly. Agglutination titer: first day, 500; third 500; fifth 700; 7th 700; 9th 1,000; 11th 1,000; 13th 1,300; 15th 1,500. In a case of pneumonia the agglutination titer rose from 300 on the day of the critical fall of temperature to 700, 10 days later, when the patient was fully convalescent. In a case of acute rhinitis with laryngitis (temperature normal) the agglutination titer rose within 19 days from 200 to 1,000.

It really seems as if any disease were capable of temporarily increasing the agglutinative power in previously vaccinated people, or of reviving it after it has disappeared. In a case of pneumonia, for instance, the agglutination test proved negative at a 1:50 dilution on the 1st and 3rd days, whereas, on the 5th day a titer of 100 was found, which ultimately rose to 300.

So far we know only of one disease which, instead of increasing or leaving unimpaired the agglutinative properties of individuals vaccinated against typhoid, causes them to disappear temporarily. It is very significant that this singular disease should be measles. This interesting exception, which so curiously confirms the well known anergic influence of measles as regards tuberculous reactions and Jennerian revaccination has been demonstrated for the first time by Leon Bernard and Paraf in 1915.

To return to our subject, I may add it matters little whether the patient has been vaccinated recently or a long time, whether he has received the full dose of vaccine or one injection only. I tabulate here some cases which show this very clearly:

TABLE IV.

Disease	Time elapsed since vaccination	No. of inoculations	Agglutinative titer
Rheumatic fever	5 months	1	600
Malaria	10 months	7	0
Amoebic liver abscess	5 months	1	400
Tonsillitis	7 months	1	300
Laryngitis catarrh	10 days	2	100
Tonsillitis	26 months	5	100
Simple diarrhea	25 months	5	200

I think the facts and figures given in this section justify the conclusion that, in individuals previously vaccinated against *B. typhosus*, the value of the agglutination test, in order to ascertain whether they are infected with typhoid or not, is absolutely nil. No caution, no niceties of technic, no careful consideration of titer or of the behavior of curves are of any avail whatever.

## II. CASES OF TYPHOID AND PARATYPHOID IN PREVIOUSLY VACCINATED INDIVIDUALS.

The cases with positive blood culture which I have studied are:

Typhoid	27
Paratyphoid A	104
Paratyphoid B	35

*Typhoid*.—All my 27 patients have given a positive Widal with *B. typhosus*. But in several instances the reaction was found negative during the whole course of actual fever and proved positive only when or after convalescence was reached, namely, for instance, on the 39th, 42d, 44th days. The maximum titers have been moderately high, ranging from 200 to 1800. In one instance the titer never went over 100, and in one other instance over 50.

Coagglutinations were relatively scarce, having been observed in five cases only, which I here tabulate:

TABLE V.

Cases	Maximum agglutination	Maximum coagglutination	
		A	B
1	1200	300	100
2	1400	900	0
3	600	400	0
4	1400	100	0
5	1000	500	500

In case No. 2, the curves of *B. typhosus* and *B. paratyphosus* A ran at about the same height during the whole course of the actual disease; it was only during convalescence that *B. typhosus* predominated distinctly.

In case No. 3 the difference between the level of both curves was very slight and gave no definite impression of a predominance of *B. typhosus*.

In case No. 5, *B. paratyphosus* A and, later on, *B. paratyphosus* B predominated very evidently, until finally the curve of *B. typhosus* took the lead.

*Paratyphoid A*.—Out of my 104 patients, 12 (11.5 p. 100) had a constantly negative test for the three germs during the whole course of their disease, none of them having been discharged from my wards before convalescence.

Not more than two patients had a positive test for *B. parat. A* only, without any coagglutination. The titer in both cases was never over 100.

Contrasting with this, 41 patients (39.5 per 100) had a positive test for *B. typh. only*, the titer being occasionally quite high (1000, 1700) and the test for *parat. A* remaining constantly negative.

In one case the test was constantly negative for parat. A and positive for both parat. B and B. typh., the latter predominating. In another case, the test was negative for B. typh. and positive for parat. A and parat. B, the former predominating slightly.

In 24 instances, parat. A and B. typh., and, in 23 instances, parat. A, parat. B and B. typh. were agglutinated. Out of those 47 cases there were only 4 in which the parat. A titer constantly predominated. In 18 cases it ultimately predominated, B. typh. or, to a lesser degree, parat. B having had the lead at the beginning of the disease. In 23 cases B. typh. predominated, namely constantly in 17 and ultimately in 6. In 2 cases, the curves were constantly intertwining and crossing each other at low level, so that no definite predomination could be made out.

It will therefore be seen that out of 104 paratyphoid A infections, with positive blood culture, 6 cases only had agglutinating properties pointing clearly to paratyphoid A (5.7 p. 100). In 18 cases the reaction was misleading during the greater part of the course of the disease, the evidence becoming clearly in favor of paratyphoid A towards convalescence only (17.3 p. 100). In 64 cases the reaction was constantly misleading, the evidence being in favor of B. typh. (61.5 p. 100). In 2 cases the reaction was doubtful all the time, no germ having a decidedly predominating titer.

I give here a tabulated resumé, where  $A=B$  or  $A=T$  means that the agglutinating titer for parat. A was practically the same as the titer for parat. B, or B. typh., and  $A>T$  means that the agglutination titer for parat. A was distinctly higher than for B. typh.

Negative altogether	12	} 14 = 13.4 p. 100.
$A=B$ or $A=T$	2	
A positive alone (B and T negat.)	2	} 6 = 5.7 p. 100.
$A>T$ or $A>B$ (constantly)	4	
$A>T$ (ultimately)	18	} 19 = 18.2 p. 100.
$A>B$ (ultimately)	1	
$T>A$ (constantly)	23	} 65 = 62.5 p. 100.
$T>B$ (A negat.)	1	
T positive alone (A and B negat.)	41	

*Paratyphoid B.*—My 35 cases give the following figures:

Negative altogether	0	} 5 = 14.2 p. 100.
$B=T$	5	
B positive alone (T and A. negat.)	0	} 5 = 14.2 p. 100.
$B>T$ (constantly)	5	
$B>T$ (ultimately)	7	} 7 = 20 p. 100.
$B>A$	0	
T B (constantly)	5	} 18 = 51.4 p. 100.
T B (ultimately)	1	
T and A positive (B negat.)	1	
T positive alone (B and A negat.)	11	

In the paratyphoid B cases, therefore, the agglutination test was unable to give any information whatsoever in 14.2 p. 100 of the cases. It gave early ac-

curate information in 14.2 p. 100, was misleading at the beginning and ultimately correct in 20 p. 100, and altogether misleading in 51.4 p. 100.

If we now sum up all our 166 previously vaccinated typhoid and paratyphoid cases, we get the following figures:

Test negative or uninterpretable	18 (10.8 per 100)
Test altogether misleading	85 (51.2 " 100)
Test first misleading, ultimately correct	25 (15.1 " 100)
Test altogether correct	38 (22.8 " 100)

It is therefore evident that, in vaccinated patients, the chances are for the agglutination test leading to error, however minute the technic, however numerous the tests. It is a remarkable thing—but not unexpected—that the error is always in one direction only: it causes us to mistake paratyphoid for typhoid, but never (as far as my experience goes) typhoid for paratyphoid. We may accordingly pretty confidently assume the diagnosis of paratyphoid A or B to be correct whenever the agglutination titer of one or the other predominates clearly and constantly during the course of the disease. However, we have seen how seldom this occurs (5.7 p. 100 of the parat. A, 14.2 p. 100 of the parat. B cases).

On the other hand, if B. typhosus only is agglutinated or if its agglutination titer predominates—and this is by far the more frequent case—no conclusion whatever can be drawn from the fact. Out of 99 charts where the curve of B. typhosus permanently predominated, 75 belonged to paratyphoid patients. This would put the probability of error to 75.7 p. 100. If we include the cases where the agglutination curve of B. typhosus predominated during the greater part of the actual course of the disease, to be superseded only later on by one of the paratyphoid curves (95 cases) we get 124 charts pointing to typhoid, among which 100 were paratyphoid. This would put the probability of error at the still higher rate of 80.6 p. 100.

### III. CASES OF TYPHOID AND PARATYPHOID IN NONVACCINATED INDIVIDUALS.

Although I can deal here only with small numbers, I think it is worth while to compare the figures obtained in the preceding section with those expressing the results of the agglutination test. In nonvaccinated typhoid and paratyphoid patients, treated and observed in the same hospital and during the same period of time.

#### TYPHOID (22 CASES)

Test negative altogether T = A or B	3 0	3 = 13.6 p. 100.
T positive alone (A and B negat.) T > B (constantly)	17 1	18 = 81.8 p. 100.
T > A (ultimately) T > B (ultimately)	1 0	1 = 4.5 p. 100.
A or B > T (constantly) A > T (constantly)	0 0	

PARATYPHOID A (12 CASES)

Negative altogether	4	}	6 = 50 p. 100.
A = B or T	2		
A positive alone (B and T. negat.)	1	}	1 = 8.3 p. 100.
A T (constantly)	0		
A > T or B (ultimately)			2 = 16.6 p. 100.
T positive alone (A and B negat.)			3 = 25 p. 100.

PARATYPHOID B (8 CASES)

Negative altogether	2	}	3 = 37.5 p. 100.
B = A	1		
B positive alone (A and T negat.)	0	}	3 = 37.5 p. 100.
B > T (constantly)	3		
T B (constantly)	1	}	2 = 25 p. 100.
T positive alone (B and A negat.)	1		

If we sum up our 42 nonvaccinated cases, including typhoid and paratyphoid, we find:

Test negative or uninterpretable	12 = 28.5 p. 100.
Test altogether misleading	5 = 11.9 p. 100.
Test first misleading, ultimately correct	3 = 7.1 p. 100.
Test altogether correct	22 = 52.3 p. 100.

If we discard the negative or uninterpretable tests, we find:

Test altogether misleading	16.6 p. 100.
Test first misleading, ultimately correct	10 p. 100.
Test altogether correct	73.3 p. 100.

In nonvaccinated patients, therefore, the rate of error is distinctly lower than in vaccinated patients. Here also the error is in one direction only: it leads to mistaking paratyphoid for typhoid, but not to the reverse. Accordingly a constantly positive and predominating parat. A or B test is a reliable argument in favor of paratyphoid, but it is a rare occurrence. On the other hand, a constantly positive T test should be considered with a great deal of caution: out of 23 such curves 5 concerned paratyphoid cases. This puts the probability of error at 21.7 p. 100.

It is interesting to remark that in nonvaccinated cases also, the error is in one direction only. It shows that vaccination against *b. typhosus* is not the only responsible factor in increasing the agglutinability of *B. typhosus* in the blood of patients infected with one of the paratyphoid germs. Even in nonvaccinated individuals, an infection with paratyphoid seems to provoke a coagglutinability for *B. typhosus* which may occasionally be of a higher titer than the specific agglutinability. That this natural tendency is considerably increased by vaccination is obvious.

CONCLUSIONS.

1. In *nonvaccinated* individuals the agglutination test is of the greatest practical value for diagnosing infection caused by the bacilli of the typhoid group.



2. But, when it comes to discriminate between the three subspecies belonging to that group, the information given by the Widal test should be accepted with great caution. A predominance of the agglutination titer of *B. parat. A* or *B* speaks almost certainly in favor of paratyphoid *A* or *B*. But if the agglutination titer of *B. typhosus* predominates, the probability of the disease being due to *B. typhosus* is only 73.3 p. 100.

3. In individuals having been previously *vaccinated* against *B. typhosus*, the agglutination test is absolutely unable to confirm a clinical diagnosis of typhoid. The behavior of the agglutination titer in vaccinated typhoid or paratyphoid patients is not distinguishable from the behavior of the same titer in vaccinated individuals suffering from any other disease.

4. In a case of clinically confirmed typhoid occurring in a previously *vaccinated* individual, the agglutination test has a practical diagnostic value whenever it shows a constant predominance of the agglutination titer for one of the paratyphoid germs. But this is a very rare finding. In the greater majority of cases the agglutinating titer for *B. typhosus* predominates. It has no diagnostic value whatever, the probability of mistaking paratyphoid for typhoid being 75.7 p. 10.

5. Blood-culture is therefore the only reliable method to ascertain whether a typhoid infection occurring in a vaccinated individual is due to *B. typhosus* or to one of the paratyphoid germs.

# ANTIBODIES IN GONOCOCCAL ARTHRITIS AFTER THE INTRA- VENOUS INJECTION OF SPECIFIC AND NONSPECIFIC PROTEIN\*

BY HARRY CULVER, M.D., CHICAGO, ILL.

WHEN a suspension of bacteria or a pure protein solution, such as primary or secondary proteose, is injected intravenously into an individual, there occurs a clinical reaction quite similar in every instance regardless of the bacteria or protein solution used, once the proper dosage is established. This reaction takes place in a normal individual as well as in one suffering from infection.

The reaction is characterized by a chill of variable severity, coming on usually within one hour following the injection, coincident with which there is, as a rule, a marked decrease in leucocytes in the peripheral circulation, as well as a drop in temperature. This condition lasts for twenty to thirty minutes, when the chill abates and the temperature of the individual gradually rises, reaching its climax in four to six hours. At this time there is usually profuse perspiration and a considerable increase of leucocytes in the peripheral circulation. The temperature and number of leucocytes in the peripheral circulation return to normal in twenty-four to forty-eight hours.

These reactions have been used, with success, in the treatment of gonococcal arthritis, epididymitis, and acute prostatitis.<sup>1</sup> In some instances of typhoid fever a critical fall of temperature, with rapid recovery of the patient, has been observed immediately following a similar reaction. This has taken place both with specific typhoid organisms and with nonspecific substances like colon bacilli and proteose solutions. Equally striking results have been seen in obscure respiratory infections associated with remittent fever.<sup>2</sup>

A pronounced feature of the action in gonococcal infections is that the most marked benefit, both subjectively and objectively, occurs within twenty-four hours following the first injection. Subsequent injections may cause some therapeutic response, which is usually not to be compared with that following the first injection.

That marked beneficial therapeutic results follow the injections in many instances of gonococcal infection there seems to be no question. The factors that should be considered in the mechanism of the improvement or recovery and which have been discussed by various investigators, are the production of fever, peripheral leucocytosis, profuse perspiration, disturbance of the ferment antiferment balance, and the mobilization of fixed antibodies.

This work was undertaken to determine if there is a specific antibody response for gonococci after the injection of nonspecific proteose solutions comparable to that which is produced by the injection of killed gonococci in gonococcal arthritis; also to determine what place, if any, antibody production or mobilization, has in the marked objective and subjective improvement seen in cases of gonococcal arthritis within 24 hours after a protein reaction.

Dreyer, Gibson and Walker<sup>3</sup> have found that paratyphoid fever B commonly

\*From the Department of Experimental Medicine University of Illinois, Chicago, Ill.

causes a rise in the typhoid agglutinin titer, frequently considerable, while paratyphoid fever A causes a less pronounced increase. Meyer and Christiansen,<sup>4</sup> by infecting a rabbit with tuberculosis noted that its serum possessed agglutinin for typhoid and the animal also gave a good typhoid intracutaneous reaction; while Conradi and Bieling<sup>5</sup> have observed that any intercurrent infection is liable to increase the typhoid agglutinin of the serum of any individual who has ever had typhoid or been vaccinated against it. Dunklin,<sup>6</sup> working with typhoid immune rabbits, noted an increase in the agglutinin for typhoid bacilli in the serum of these animals after an intravenous injection of proteose solution. Ludke<sup>7</sup> on the other hand, states that there is no change in the agglutinin content of serum of typhoid patients, for typhoid bacilli, after the intravenous injection of secondary proteose.

Bull<sup>8</sup> found that within twenty-four hours after an intravenous injection of killed typhoid bacilli in normal and typhoid immune rabbits, there is an increase in lytic substances for typhoid bacilli in the serum. Teague and McWilliams<sup>9</sup> repeated and extended Bull's work and conclude that the bacteriolytic power of the serum of normal and immune rabbits for typhoid bacilli is neither increased nor decreased by a large intravenous injection of killed typhoid bacilli, while the serum of a rabbit that has received only one or two immunizing injections shows a distinct but not a great increase in its bactericidal power within twenty-four hours after such an injection.

The opsonins and bactericidal substances were studied in the serum of sixteen patients suffering from gonococcal arthritis. An injection of killed gonococci or proteose solution was given intravenously every seventy-two to ninety-six hours until three to five injections had been given. The serum was taken for study just before each injection. Ten of these patients received successive injections of proteose and six received killed gonococci. No marked difference was seen in the results of the two groups.

The opsonin content was determined by the extinction method as described by Klein.<sup>10</sup> The bactericidal substance in the serum was determined by the dilution method; i.e., the bactericidal titer of the serum is represented by that dilution of the serum which causes an appreciable decrease in the number of colonies upon plating, from the different dilutions, at successive intervals, a normal control serum being always used at the same time. Teague and McWilliams<sup>9</sup> and before them Buxton<sup>11</sup> concluded that the bactericidal titer of an immune serum for typhoid bacilli as determined by the usual dilution method, with or without the addition of complement, is not an indication of the bactericidal power of the blood plasma *in vivo*. However, with so delicate an organism as the gonococcus, this method gives more regular and more readily comparable results than by the use of a method in which a standard amount of serum is used for a standard time in contact with a known constant number of gonococci and the action of the serum determined by plating at regular intervals. Since only three to five successive injections were given each patient, the factor of a too highly immune serum being developed, seldom interfered. If, however, after several successive injections it was found that the patient's serum would fail to kill in concentrated serum solution, but would kill in less concentrated solutions, it was invariably found that the killing zone had moved

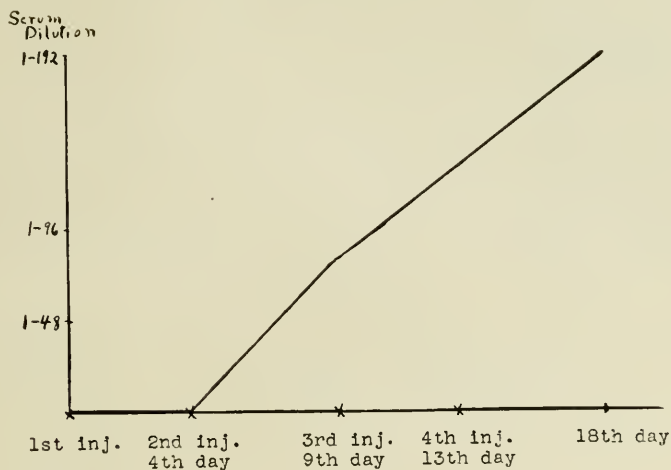


Chart 1.—Oponin curve after the intravenous injection of killed gonococci.

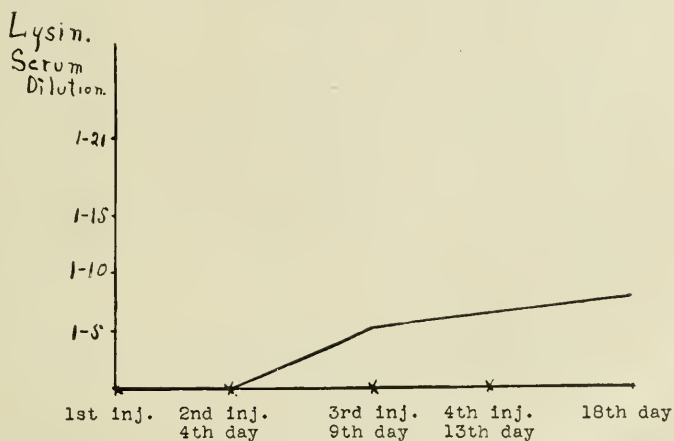


Chart 2.—Lysin curve of same serum as represented in Chart 1.

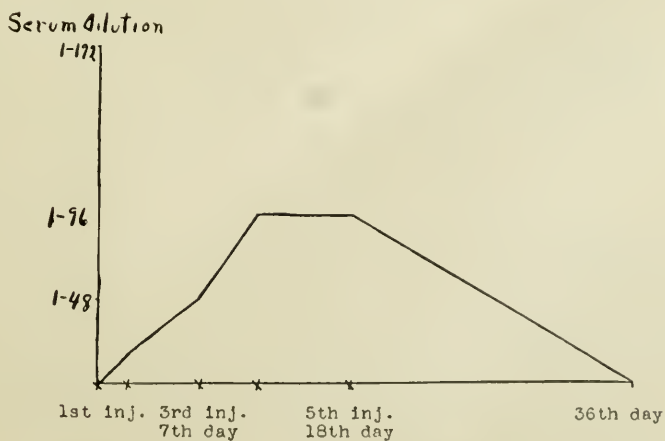


Chart 3.—Oponin curve after the intravenous injection of secondary proteose.

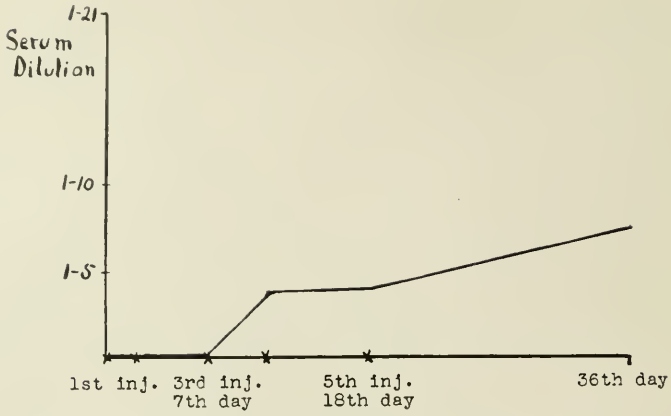


Chart 4.—Lysin curve of the same serum as represented in Chart 3.

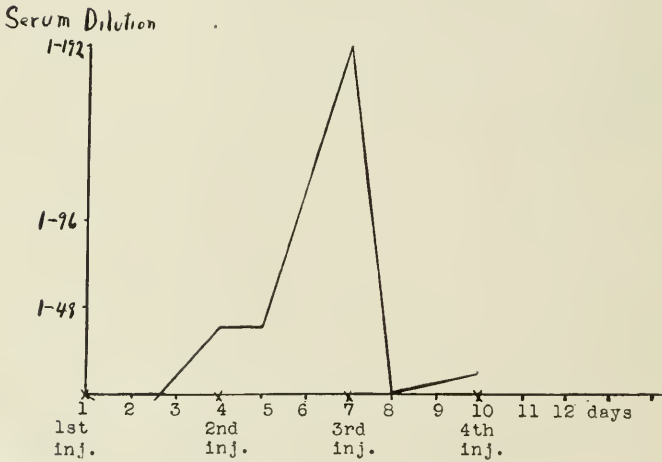


Chart 5.—Opsonin curve of serum taken just before, and 24 hours after, successive intravenous injections of killed gonococci.

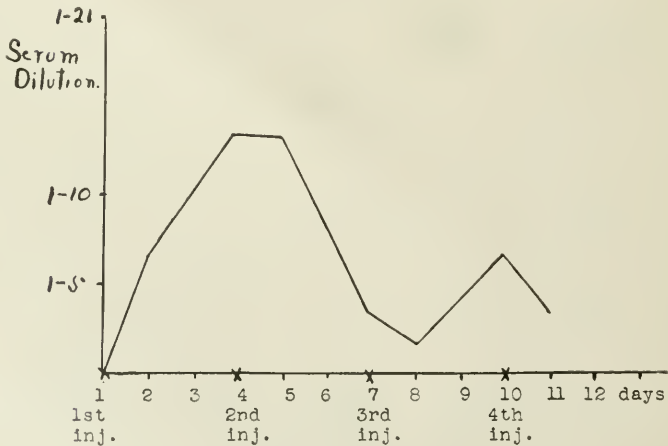


Chart 6.—Lysin curve of the same sera as represented in Chart 5.



to a serum with a greater dilution than before the pro-killing zone developed. In these instances the bactericidal titer is considered the highest dilution of the killing zone.

The serum to be tested was successively diluted with nutrient broth. To a standard amount of each serum dilution was added a drop of a standard suspension of gonococci twenty-four hours old. A loop of material from each tube was plated at once and serum mixtures incubated at 37° C. for four hours when a second loop of material was plated from each dilution. The plates were

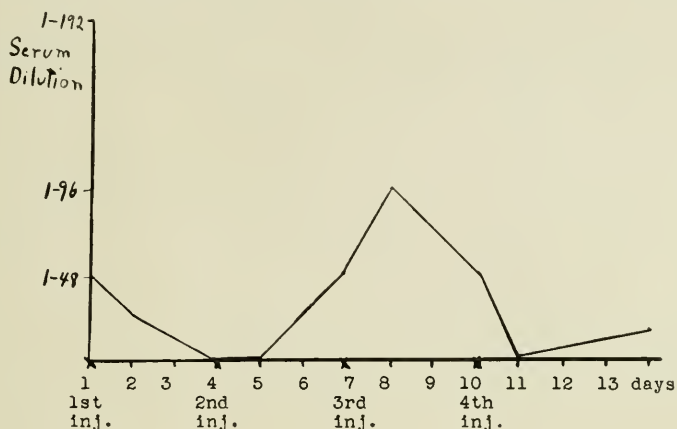


Chart 7.—Opsonin curve of serum taken just before, and 24 hours after, successive intravenous injections of primary proteose.

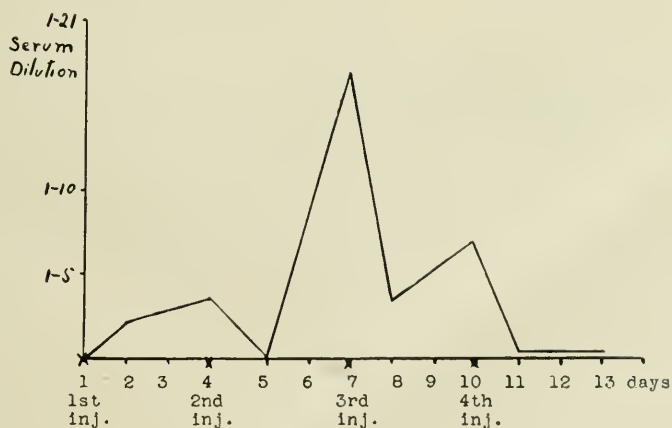


Chart 8.—Lysin curve of the same sera as represented in Chart 7.

incubated for forty-eight hours and then all colonies were counted on each plate. A comparison of these results was then made to determine the highest dilution in the killing zone. It was found at the beginning of this work that the fresh serum to be tested was sufficiently complemented to cause bacteriolysis even in the highest dilution 1:64. Hence it was not necessary to inactivate and reactivate each dilution with a known amount of complement.

Antibody response after injections of specific substances is known to be very variable in different individuals and in the same individual at different times.

After a careful study of sixteen patients, however, there was noted no appreciable difference between the antibody production following killed gonococci injections and proteose solution injections. This fact can best be shown graphically in any two cases as in Charts 1, 2, 3, and 4. These charts clearly show the increase in opsonin and lysin of the patient's serum for gonococci after the injection of dead gonococci and proteose solutions. There is no distinguishing feature in these curves by which the injected material could be recognized. The opsonin reached the highest point after the injection of gonococci, while the lysin curve rises after the injection of proteose. However, these conditions may be reversed with any other two cases similarly traced. This antibody response is variable but present to some degree in every patient of the sixteen injected, whether the material injected was specific gonococci or nonspecific proteose and also regardless of the therapeutic response obtained.

In a second series of six patients, three were treated with injections of killed gonococci and three with primary and secondary proteose solutions, the serum of each being tested just before and twenty-four hours after each injection, at which time the most pronounced therapeutic effect is present. Charts 5, 6, 7 and 8 show the antibody curves for two typical cases and represent the usual findings of a slight increase in lytic substance following the first injection, whether gonococci or proteose and a corresponding decrease in opsonin. All succeeding injections cause either a decrease or no change at all in both lysin and opsonin during the first twenty-four hours following the injections. An unusual condition occurred in Chart 8 where there is seen a moderate rise in opsonin within twenty-four hours following the third injection of proteose.

#### COMMENT.

By the methods above outlined it was found that both the opsonic and bacteriolytic titer of the serum of patients suffering from gonococcal arthritis were approximately normal or below before any injections were made. This has been previously observed by Irons<sup>12</sup> and others for the opsonin content of serum from these patients. He also demonstrated that spontaneous fluctuations in antibody content occur in gonococcal arthritis and that by massage of the affected joints or the infected prostate there resulted an opsonin increase not altogether unlike that produced by an injection of killed gonococci. Therefore any antibody change which results from an injection of a substance which produces a chill might well be explained by the motion of the affected parts during the chill. Since the curves regularly show an increase in lysin and a decrease in opsonin after the first injection and reaction, while each succeeding injection is usually followed directly by an antibody decrease regardless of the intensity of the reaction, it does not seem that the joint motion has much to do with it here. The above antibody changes occur in serum of patients with acute, subacute, or chronic joints with no appreciable difference between these classifications; which might be expected were the antibody changes due entirely to motion of the affected parts. Some sera have been studied after a small amount of protein was injected, a large enough amount to increase the leucocytes and temperature but not sufficient to produce rigor. These injections were followed by antibody changes not unlike those produced by the more severe reactions.

## SUMMARY.

Primary and secondary proteose preparations stimulate antibody production or mobilization for specific organisms in gonococcal arthritis, in a manner not to be distinguished from that produced by the injection of the specific organisms themselves.

In gonococcal arthritis, there is either no change or a decrease in the antibody content of serum within the first twenty-four hours following an intravenous injection, in all excepting the first injection when the lytic substances seem to be slightly increased during this time.

In favorable patients the first injection usually causes the greatest clinical benefit. However, refractory patients may give a similar lysin increase during the first twenty-four hours following an injection; hence the subjective and objective improvement in favorable cases can not unquestionably be attributed to an increase in antibodies alone. However, little as these substances may have to do with the early relief of symptoms in gonococcal arthritis, it may be that they have considerable to do with the final recovery from the infection by their influence upon the primary focus.

It would seem that there is no one particular factor which is responsible for the benefit derived in favorable patients, but a series of events occur, which, when acting together or in succession, tend toward the relief and ultimate recovery of the individual from the infection.

## BIBLIOGRAPHY.

- <sup>1</sup>Culver: Jour. Am. Med. Assn., 1917, lxxviii, 362.
- <sup>2</sup>Petersen, W. F.: Personal communication.
- <sup>3</sup>Dreyer, Gibson and Walker: Lancet, London, 1916, cxc, 766.
- <sup>4</sup>Meyer and Christiansen: Jour. Infect. Dis., 1917, xx, 391.
- <sup>5</sup>Conradi and Bieling: Deutsch. med. Wchnschr., 1916, xlii, No. 42.
- <sup>6</sup>Cited by Jobling and Petersen: Jour. Am. Med. Assn., lxvi, 1753.
- <sup>7</sup>Ludke: München med. Wchnschr., 1915, lxii, 321.
- <sup>8</sup>Bull: Jour. Exper. Med., 1916, xxiii, 419.
- <sup>9</sup>Teague and McWilliams: Jour. Immunology, 1917, ii, 167.
- <sup>10</sup>Klein: Bull. Johns Hopkins Hosp., 1907, xviii, No. 196, 245.
- <sup>11</sup>Buxton: Jour. Med. Research, 1904-05, xiii, 431.
- <sup>12</sup>Irons: Jour. Infect. Dis., 1908, v, 279.

## OBSERVATIONS ON THE BLOOD AND URINE AMMONIA IN ACIDOSIS\*

BY H. L. McNEIL, M.D., AND M. D. LEVY, M.D., GALVESTON, TEXAS.

THE following investigation was undertaken as a result of the accidental discovery of an unusually high ammonia content of the blood in a male patient, a chronic alcoholic, who was suffering from repeated periodic attacks of nausea and vomiting of unknown etiology, the attacks having commenced without any apparent cause. Although the etiology of the vomiting in this case was unknown, the fact that the patient, a man fifty years of age, was a chronic user of whiskey in rather large amounts suggested some connection between this fact and his symptoms. There was no evidence of nephritis or of any other pathologic lesions to explain his symptoms. (This case has elsewhere been reported in detail.<sup>1</sup>)

During the course of routine analyses of the blood of this patient for urea, an estimation of his blood ammonia was made, which showed an ammonia content of 23 mg. of ammonia per 100 c.c. of blood. Suspecting an error in technic as the explanation of this high ammonia content, numerous subsequent estimations of blood ammonia were made on different days, all of which showed a marked increase. These ammonia estimations were made after the aeration method of Folin, using 1/50 N. sulphuric acid to absorb the ammonia and titrating this against 1/50 N. sodium hydroxide with alizarin as an indicator. As a further control on these estimations numerous similar estimations were carried out on other patients at the same time, as well as on seven normal individuals, and in none of them was any abnormal amount of blood ammonia demonstrated. A reference to the protocol will show that on four subsequent occasions 16, 12, 8, and 3 mg. of blood ammonia were found, while as the condition of the patient gradually improved other estimations showed that the excess of blood ammonia had disappeared.

A search of the literature upon the subject of blood ammonia in normal and diseased conditions reveals comparatively few satisfactory studies. Since the more accurate methods of estimating ammonia in the blood are comparatively recent, one can accept only with great reservation the observations made before the methods of Folin were introduced. A few apparently authentic, isolated instances of increased blood ammonia in human beings have, however, been reported in cases of acidosis. In these cases, practically none of which are given in sufficient detail to be of much real value, it was believed that the blood ammonia increase was a compensatory phenomenon resulting from the increase in the acid radicals of the blood during acidosis. A number of isolated cases are also mentioned in the literature in which an increase of blood ammonia was found in association with eclampsia, uremia, acute yellow atrophy of the liver, phosphorus poisoning, and in the terminal stages of cirrhosis of the liver. In practically all of these instances, however, the ammonia increase was ascribed to acidosis. On the other hand, numerous careful studies upon the chemistry

\*From the Department of Internal Medicine, University of Texas Medical School, Galveston, Texas.



of the blood in such conditions have failed to show any constant increase in blood ammonia although an increased ammonia coefficient of the urine is known to be commonly present in certain diseases of the liver, as in some cases of eclampsia, in acute yellow atrophy of the liver, and the pernicious vomiting of pregnancy.

In this connection the well-known work of Pawlow and his associates<sup>2</sup> done many years ago upon Eck fistula dogs, which showed symptoms of toxemia upon high protein diets, is of interest. While the observations of these observers is now somewhat discredited so far as their studies of the blood ammonia in these toxic animals is concerned, the methods which they used for estimating ammonia having been open to question, nevertheless, their claims as to increased ammonia percentages in blood and urine of these intoxicated animals is of interest. Fischler<sup>3</sup> has also, more recently, put forward similar claims in accounting for protein intoxications in Eck fistula dogs whose livers have been thrown out of the portal circulation, basing his claims, however, upon an excess of ammonia in the urine rather than the blood.

In the present state of our knowledge regarding the relation of increased blood ammonia to disease, a study of the literature would, in short, suggest that a condition of acidosis is usually present when such increases are noted. The same explanation would be the apparent explanation also, for increases of ammonia excretion in the urine in most cases, although the work of Williams,<sup>4</sup> Losee and Van Slyke,<sup>5</sup> and others would seem to indicate that an *increased ammonia coefficient in the urine* may occur in the pernicious vomiting of pregnancy without the accompanying acidosis.

#### BLOOD AMMONIA CONTENT IN NORMAL INDIVIDUALS.

As has been stated, the observations upon the blood ammonia of both human beings and animals, carried out by the older observers, are open to question due to the more or less inaccurate technic. The development of the methods suggested by Folin<sup>6</sup> have, however, enabled the observer to carry out blood ammonia estimations in a comparatively accurate manner. All recent observers, using Folin's methods or a modification of the same, are now apparently agreed that the blood ammonia in normal men and animals never exceeds 1 mg. per 100 c.c. of blood. In fact 1 mg. per 100 c.c. may be considered as the extreme upper limit for blood ammonia. Medwedew<sup>7</sup> has, for example, found an average content of 0.56 mg. per 100 c.c. in normal dogs. Rhode<sup>8</sup> found the ammonia content of dogs to vary between 0.17 and 0.72 mg. per 100 c.c. Gettler and Baker<sup>9</sup> found an average of between 0.4 and 0.75 mg. per 100 c.c. of blood in 30 normal human beings. Bennett,<sup>10</sup> using a different technic, found an average blood ammonia content in five normal men of only between 0.07 and 0.1 mg. per 100 c.c. Finally Folin and Denis<sup>11</sup> have always found less than 1 mg. per 100 c.c. in both men and animals as the ammonia content of the systemic blood.

In carrying out routine examinations upon the blood for its ammonia content, however, it is necessary to bear in mind the very important fact that blood after its withdrawal quickly shows an increase in its content of ammonia, apparently due to the decomposition of certain nitrogenous bodies which are normally present in it. Accordingly, unless estimations for ammonia are made promptly after the withdrawal of blood, the real values of ammonia can not be

obtained. That the production of ammonia in blood which is allowed to stand after being withdrawn must be considered as an important factor in such estimations is shown by the work of Medwedew,<sup>7</sup> who found that after standing for 24 hours, the ammonia content of the blood of dogs would rise from normal to as high as 2.93 mg. per 100 c.c. It was shown, moreover, that the ammonia formation began within a few minutes after the withdrawal of the blood. This observation has since been confirmed by Folin and Denis, Rhode,<sup>8</sup> and others. During the course of our own investigations, we also have found the same to hold true of human blood. In no instances, however, have we found the ammonia content of sterile normal blood to rise above 3 mg. per 100 c.c., even after prolonged standing, nor have any of the observers mentioned above found the rise in ammonia to exceed this limit after standing. Apparently, therefore, the normal upper limit for ammonia in perfectly fresh blood may be taken as 1 mg. per 100 c.c., and under any circumstances, with a proper technic, a finding of over 3 mg. per 100 c.c. may be safely taken as abnormal.

#### TECHNIC OF ESTIMATIONS.

In the following studies an effort has been made to make the technic as uniform as possible. The blood has been examined immediately after its withdrawal into thoroughly cleansed tubes, to which was added 1 c.c. of a 2 per cent citrate solution to prevent clotting. The aeration method of Folin was used, brisk aeration for one-half an hour being applied, and the ammonia which was blown over being collected in a 1/50 N. solution of sulphuric acid. This was then titrated from a microburette with 1/50 N. sodium hydroxide, using an aqueous solution of alizarin as an indicator. Since, from a clinical standpoint, only comparatively marked variations in any chemical element of the blood can be considered of any practical diagnostic significance, an attempt was made in these observations to detect only those increases of ammonia which exceeded 1 mg. per 100 c.c. of blood, and in the following tables all amounts of 1 mg. and under are expressed as a "trace." Also, all amounts up to 3 mg. per 100 c.c. have been considered as possibly due to changes attributable to variations in technic, although it is our belief that the technic was not responsible for such increases.

In order to remove, as far as possible, the factor of diet from the results of these examinations, an effort was made to place all patients, whenever possible, upon a diet consisting of 32 ounces each of milk and albumin water per 24 hours for at least 24 hours preceding the examination, the above diet constituting the ordinary hospital liquid diet. Our experiments upon normal individuals would seem to show, however, that at least in normals, diet has no influence upon ammonia increases since, of our seven normal individuals, all of whom showed less than 1 mg. per 100 c.c.; all were purposely placed upon high protein diets preceding the tests.

The alveolar air estimations for CO<sub>2</sub> tension were carried out after the method of Fridericia.<sup>12</sup> Acetone and diacetic acids were estimated by the sodium nitroprusside and the ferric chloride tests, respectively. The bicarbonate test of Sellards<sup>13</sup> for acidosis was considered positive when it required more than 15 gm. of bicarbonate taken by mouth to alkalinize the urine. The blood urea

estimations were carried out after the method of Van Slyke and Cullen, using Marshall's urease method to decompose the urea. Five cubic centimeters of blood each were used in the estimations of both urea and ammonia.

#### DISEASES STUDIED.

Our findings as to the blood ammonia content in various pathologic conditions may best be considered, perhaps, by a consideration of the diseases in separate groups. The clinical diagnoses in the following cases have been made as carefully as possible by modern clinical methods and in a considerable percentage of cases the diagnoses have been confirmed by autopsy.

#### NEPHRITIS.

As will be seen from the protocol, 19 cases of nephritis have been studied. Among these cases are included the various types of nephritis which are met with clinically, both the high tension cases with moderate edema and those types characterized by general anasarca with only slight urea retention being represented. In this series are included also, four cases dying, of uremia, as well as several mild cases. As will be seen from the protocol, three cases in this series showed increases in blood ammonia which might be considered marked (over 3 mg. per 100 c.c.). It is interesting to note that none of the four uremics showed such blood ammonia increases. It is also of note that all three of the cases showing an excess of blood ammonia were also complicated by myocarditis with definite evidence of cardiac failure and with chronic passive congestion of the visceral organs. All three of these cases showed definite evidence of acidosis.

#### CARDIAC FAILURE.

In addition to the cases of cardiac disease included in the nephritic series, fourteen other cardiac cases were studied, not complicated by nephritis. Four of these showed blood ammonia in quantities of over 3 mg. per 100 c.c. It is of interest to note that all of these cases also showed evidence of acidosis, and that all were suffering from chronic passive congestion of rather marked degree, several having pulsating livers. It should be mentioned that while all of the four cases showing blood ammonia increases in this series, also gave evidence of acidosis, nevertheless, several other cases in which the acidosis was just as severe failed to show blood ammonia increases.

#### CHRONIC DISEASES OF THE LIVER.

In view of the close relation supposed to exist between the liver and the normal metabolism of the proteins, and in view of the apparently close connection between the liver and the conversion of the ammonia formed by protein metabolism into urea, one would expect theoretically to find evidence of accumulation of ammonia in the blood in serious diseases of that organ. As a matter of fact, however, reference to the protocols will show that such is apparently not the case, at least in chronic diseases of the liver. In only one of such cases studied in this series has any definite increase of blood ammonia been found, this being a case of syphilis of the liver, with severe toxemia, whose blood was



examined just before death. In this series, as will be noted, were included four cases of Laennec's cirrhosis of the liver, three cases of syphilitic cirrhosis, one case of amebic abscess of the liver, and two cases of rather marked jaundice. Chronic diseases of the liver may be said, therefore, to have no definite and constant connection with increase in blood ammonia.

#### TOXEMIAS OF PREGNANCY.

In connection with disease of the liver the few observations which we have been enabled to carry out upon several forms of toxemias occurring during pregnancy may be of interest, since at least certain forms of such toxemias, such as the pernicious vomiting of pregnancy and eclampsia are well known to be associated with acute hepatic lesions, and with increased ammonia coefficients in the urine, as was first emphasized by Williams.<sup>4</sup> In this series three cases diagnosed clinically as probable cases of eclampsia, and one case suspected of being the pernicious vomiting of pregnancy, were studied. It is interesting to note that two out of three of the cases diagnosed as eclampsia showed definite blood ammonia increases, while one case of persistent vomiting complicating pregnancy showed a somewhat less marked increase. It is also of interest that one of these cases, showing an increase in blood ammonia, failed to show any evidence of acidosis. Also, one case of this series, which had shown a marked increase in blood ammonia, coming to autopsy, showed the typical hepatic lesions of eclampsia.

#### ACIDOSIS.

Thirteen cases of acidosis from various causes other than cardiorenal disease have been studied in this series. The majority of these cases were characterized by the severity of the acidosis. Six of them were diabetics. One was a case of diabetic coma. In spite of the fact that most of these cases of acidosis were characterized by their severity as determined by their symptoms and by the usual tests for acidosis, one is surprised to note the small percentage of cases showing marked increases in their blood ammonia. Only two out of thirteen cases showed marked blood ammonia increases. Both were postoperative cases, one being a case of general peritonitis, which at autopsy showed a rather marked cloudy swelling of all organs, and in addition, showed marked fatty degeneration of the liver. The other case was one of persistent nausea and vomiting following a laparotomy. The nausea and vomiting at the time of the blood examination had persisted for three weeks, and was evidently not the ordinary type of postoperative vomiting. The vomiting in this case was suspected of being of nervous origin, although it was severe enough to cause a rather serious condition clinically. The patient recovered, however. It is of interest in connection with these cases of acidosis, to note that blood ammonia is apparently by no means proportional quantitatively to the severity of the acidosis, since the severest cases of acidosis noted both in the cases included under acidosis, as well as in the cases of acidosis complicating heart and kidney diseases, were not as a rule the ones showing the highest blood ammonia content. Moreover, it is of interest that the single case of diabetic coma with unusually severe acidosis, studied in this series, failed to show any marked increase in blood ammonia, although the *urine*



did contain a marked excess of ammonia. The well-known fact regarding the relation of acidosis to an increased content of ammonia in the urine was also confirmed in those cases of acidosis in our series, in which 24-hour urine specimens could be obtained.

#### DISCUSSION.

As stated above, from our observations, there would seem to be no definite connection between the severity of the acidosis and the high ammonia content of the blood, although the connection between acidosis and the high content of the urine ammonia is evident. Moreover, at least two cases observed by us, whose blood contained an excess of ammonia, failed to show any definite evidence of acidosis as determined by the usual tests, as well as by clinical signs.

In Case No. 69, which showed a rather large percentage of urine ammonia without a corresponding increase of blood ammonia, we have made an effort to determine, if possible, the reason for this discrepancy. In this case the ureters of each kidney were catheterized, and the fresh urine, immediately after its excretion from the kidney, was analyzed for ammonia. In this case, a case of severe diabetic acidosis, to our surprise, the smallest amount of ammonia was found in the absolutely fresh urine, only 5 mg. per 100 c.c. being noted, whereas the 24-hour specimen of urine had shown 210 mg. of ammonia per 100 c.c. (24-hour specimen preserved with toluol). In another case also (No. 100), which showed an increased blood ammonia percentage, as well as an increased ammonia output in the urine, a fresh catheterized specimen of urine from the ureter showed only 13 mg. of ammonia per 100 c.c. as contrasted with a content of the 24-hour specimen of urine, collected according to the usual precautions (toluol) of 330 mg. per 100 c.c.

Two explanations for the differences between the blood ammonia percentages and the ammonia content of the urine in acidosis suggest themselves. One explanation is that the ammonia which is believed to be set free into the blood in order to combine with, and thus neutralize, the excess of acid radicals which are present in acidosis, is set free and excreted in waves, as it were, the patient showing at one time an excess of blood ammonia and at another time showing a normal ammonia content. If this is the case, any excess of ammonia in the blood, whether the ammonia be free or combined, must be excreted by the kidneys, almost as soon as it is produced. Against this theory is the fact that even repeated examinations of the blood of some of our cases of severe acidosis have failed to show any excess at any time. Another explanation for the marked excess of ammonia in the urine in cases of acidosis, with an absence of such excess in the blood, may be, that in acidosis certain bodies are present in the urine which favor a more rapid decomposition of its nitrogenous elements than under normal conditions.

The cause of increased ammonia content of the blood without an acidosis being present presents an interesting question. Three such cases have been observed in this series: case No. 4, in which every test for acidosis was negative, including the hydrogen-ion concentration of the blood; case No. 99, whose alveo-

lar air tension, while slightly lower than normal would not suggest any appreciable degree of acidosis; and case No. 109, showing also only a slightly lowered  $\text{CO}_2$  tension. Apparently acidosis as a cause of the ammonia increase can be ruled out in the above cases. An analysis of the cardiorenal cases, showing increased blood ammonia, shows that in addition to the acidosis which was present in all, a condition of chronic passive congestion was also present in all of them, in addition to the acidosis. Moreover, in all of these cases the chronic passive congestion was quite severe. On the other hand, however, a number of cases showing similar chronic passive congestion failed to show an increased blood ammonia percentage. In fact it may be said that the cases of chronic passive congestion showing definite increases of blood ammonia are exceptional, as judged from the cases included in this series, as well as from a number of similar cases studied by us, but not included. A pathologic study of five cardiorenal cases which have come to autopsy and which have shown increased blood ammonia before death, has revealed the typical lesions of marked chronic passive congestion with pressure atrophy about the central veins of the hepatic lobules in each case. The source of the ammonia excess in the blood in the cases of eclampsia studied by us is an interesting subject for speculation. Has it any connection with the hepatic lesions which were shown to be present in at least one of these cases which came to autopsy? Our own series of such cases is too small to determine this point. If it be true, however, that the increase of blood ammonia in such cases may be attributable to the hepatic lesions, a very important differential criterion between hepatic and nephritic toxemia of pregnancy would be offered.

Also in the case of persistent vomiting in a man (case No. 4 of the protocol) in which no evidence of acidosis could be demonstrated in spite of the great increase of blood ammonia, can one explain the clinical symptoms as well as the chemical abnormality of the blood by acute hepatic insufficiency? Certainly his symptoms were similar in many respects to those exhibited by dogs showing evidence of protein intoxication after their livers have been removed from the portal circulation by means of Eck fistulas. Recent work has, however, failed to show any ammonia excess in the blood of such dogs, and Fiske and Karsner have failed in an attempt to produce blood ammonia excess in the blood of animals whose livers have been injured by poison.<sup>11, 14, 15, 16</sup> Also, against disease of the liver as an explanation of blood ammonia increase in these cases, we have studied a series of ten cases of more or less serious disease of the liver, in only one of which, just before death, was any increase of blood ammonia demonstrated. While it is true that an isolated case of marked chronic passive congestion of the liver has occasionally shown an increase in blood ammonia, such cases are, nevertheless, exceptional. Certainly, if ammonia increase in the blood is to be attributed to deficient functional activity of the liver, it must be attributed by a sudden or acute stoppage of the normal function of the cells rather than to either acute or chronic inflammatory diseases of the liver parenchyma as in cirrhosis of the liver, hepatic abscess, etc., in which either compensatory changes must have taken place in the liver or else other organs have been enabled to take up at least a part of its functions.

TABLE I.  
CHRONIC DISEASES OF THE LIVER.

SERIAL NUMBER	BLOOD NH <sub>3</sub> (MG. PER 100 C.C.)	URINE NH <sub>3</sub> (MG. PER 100 C.C. AND OUTPUT IN GM. IN 24 HRS.)	BLOOD UREA (MG. PER 100 C.C.)	URINE UREA (OUTPUT IN GM. IN 24 HRS.)	CO <sub>2</sub> TENSION OF ALVEOLAR AIR. (MM. OF HG.)	BICARBONATE TEST. (SEL- LARD'S TEST.)	ACETONE AND DIACETIC ACID IN URINE	DIAGNOSIS	REMARKS
15	Trace	50 mg. per 100 c.c. (0.49 gm. in 24 hrs.)	46 mg. per 100 c.c.	7.2 gm. in 24 hrs.	37.8 mm.	Positive	None	Laennec's cirrhosis of the liver	Complicated by diarrhea.
45	1.2 mg. per 100 c.c.	45 mg. per 100 c.c. (0.51 gm. in 24 hrs.)	89 mg. per 100 c.c.	5 gm. in 24 hrs.	38 mm.	Negative	None	"	Complicated by chronic diffuse nephritis and ascites.
106	Trace	-	39 mg. per 100 c.c.	-	44 mm.	-	None	"	"
82	2.4 mg. per 100 c.c.	60 mg. per 100 c.c. 0.5 gm. in 24 hrs.	328 mg. per 100 c.c.	2.4 gm. in 24 hrs.	34.2 mm.	-	None	"	Complicated by chronic diffuse nephritis and uremia.
84	Trace	-	29 mg. per 100 c.c.	-	41 mm.	Negative	None	"	Complicated by hematemesis.
59	Trace	-	23 mg. per 100 c.c.	-	40.2 mm.	-	-	Syphilitic cirrhosis of the liver	Death in coma. (Autopsy.)
110	Trace	-	26 mg. per 100 c.c.	-	37 mm.	-	-	"	Death from malignant endocarditis. (Autopsy.)
109	6.8 mg. per 100 c.c.	-	22.6 mg. per 100 c.c.	-	38 mm.	Negative	None	"	Blood taken just before death during coma. Death occurred in convulsions.
19	Trace	-	10 mg. per 100 c.c.	-	37.8 mm.	-	+	Carcinoma of head of pancreas	Marked obstructive jaundice. Emaciation marked.
23	Trace	-	15.2 mg. per 100 c.c.	-	47 mm.	-	-	Catarrhal jaundice (acute)	
111	Trace	-	-	-	-	-	-	Amebic abscess of liver	Large amebic abscess.

TABLE II.  
DISEASES OF THE HEART.

SERIAL NUMBER	BLOOD NH <sub>3</sub> (MG. PER 100 C.C.)	URINE NH <sub>3</sub> (MG. PER 100 C.C. AND OUTPUT IN GM. IN 24 HRS.)	BLOOD UREA (MG. PER 100 C.C.)	URINE UREA (OUTPUT IN GM. IN 24 HRS.)	CO <sub>2</sub> TENSION OF ALVEOLAR AIR. (MM. OF HG.)	BICARBONATE TEST. (SEL- LARD'S TEST.)	ACETONE AND DIACETIC ACID IN URINE	DIAGNOSIS	REMARKS
78	Trace	—	35 mg. per 100 c.c.	—	19 mm.	—	None	Myocarditis	Acute dilatation. Marked dyspnea. Chronic passive congestion marked. Autopsy.
14	Trace	30 mg. per 100 c.c. 0.3 gm. in 24 hrs.	65 mg. per 100 c.c.	—	19 mm.	—	+	"	Cheyne-Stokes respiration. Chron- ic passive congestion marked. (Autopsy.)
24	8.5 mg. per 100 c.c.	40 mg. per 100 c.c. (0.4 gm. in 24 hrs.)	22 mg. per 100 c.c.	6 gm. in 24 hrs.	25 mm.	—	+	Aneurysm, myo- carditis	Chronic dilatation. Marked dysp- nea. Marked chronic passive congestion. (Autopsy.)
31	Trace	54 mg. per 100 c.c. (0.6 gm. in 24 hrs.)	20 mg. per 100 c.c.	7 gm. in 24 hrs.	39 mm.	—	None	Endocarditis	Aortic insufficiency (compensated partially).
38	Trace	34 mg. per 100 c.c. (0.3 gm. in 24 hrs.)	40 mg. per 100 c.c.	7.5 gm. in 24 hrs.	42 mm.	—	None	"	Compensated valve lesion.
43	Trace	—	38 mg. per 100 c.c.	—	20.5 mm.	—	+	"	Aortic insufficiency. Marked dysp- nea. Marked chronic passive congestion.
48	Trace	75 mg. per 100 c.c. (0.32 gm. in 24 hrs.)	23 mg. per 100 c.c.	4 gm. in 24 hrs.	44 mm.	—	None	Myocarditis	Compensated valvular lesion.
49	Trace	50 mg. per 100 c.c. (0.45 gm. in 24 hrs.)	38 mg. per 100 c.c.	7.2 gm. in 24 hrs.	—	—	None	Endocarditis	Chronic dilatation. Marked dysp- nea. Marked chronic passive congestion.
50	6 mg. per 100 c.c.	50 mg. per 100 c.c. (0.5 gm. in 24 hrs.)	34 mg. per 100 c.c.	6.5 gm. in 24 hrs.	20 mm.	—	+	Myocarditis	Slight dilatation. Dyspnea. Chron- ic passive congestion.
58	Trace	—	21 mg. per 100 c.c.	—	—	—	+	Myocarditis	Chronic dilatation. Marked dysp- nea. Marked chronic passive congestion.
76	3 mg. per 100 c.c.	—	50 mg. per 100 c.c.	—	22 mm.	—	+	Myocarditis	Chronic dilatation. Marked dysp- nea. Marked chronic passive congestion.
35	17 mg. per 100 c.c.	130 mg. per 100 c.c. (1.2 gm. in 24 hrs.)	25 mg. per 100 c.c.	9.5 gm. in 24 hrs.	28 mm.	—	+	Myocarditis	Chronic dilatation. Marked dysp- nea. Marked chronic passive congestion. (Autopsy.)
90	3 mg. per 100 c.c.	—	24 mg. per 100 c.c.	—	31 mm.	—	+	Myocarditis	Acute dilatation. Marked chronic passive congestion.
71	4 mg. per 100 c.c.	—	37 mg. per 100 c.c.	—	28 mm.	—	—	Myocarditis	Chronic dilatation. Marked chron- ic passive congestion. Dysp- nea. (Autopsy.)

TABLE III.  
ACIDOSIS FROM VARIOUS CAUSES.

SERIAL NUMBER	BLOOD NH <sub>3</sub> (MG. PER 100 C.C.)	URINE NH <sub>3</sub> (MG. PER 100 C.C. AND OUTPUT IN GM. PER 24 HRS.)	BLOOD UREA (MG. PER 100 C.C.)	URINE UREA (OUTPUT IN GM. PER 24 HRS.)	CO <sub>2</sub> TENSION OF ALVEOLAR AIR. (MM. OF HG.)	BICARBONATE TEST. (SEL- LARD'S TEST.)	ACETONE AND DIACETIC ACID IN URINE	DIAGNOSIS	REMARKS
52	Trace	60 mg. per 100 c.c. (0.48 gm. in 24 hrs.)	29.5 mg. per 100 c.c.	9.75 gm. in 24 hrs.	42 mm.	+	++	Diabetes mellitus	Sugar free following starvation.
54	Trace	110 mg. per 100 c.c. (2.5 gm. in 24 hrs.)	-	-	33 mm.	+	++	"	"
55	Trace	40 mg. per 100 c.c. (0.78 gm. in 24 hrs.)	29 mg. per 100 c.c.	-	46 mm.	-	Slight amounts	"	"
69	Trace	210 mg. per 100 c.c. (5 gm. in 24 hrs.)	22 mg. per 100 c.c.	8.2 gm. in 24 hrs.	18 mm.	++	+++	"	Diabetic coma.
102	Trace	90 mg. per 100 c.c. (1.8 gm. in 24 hrs.)	-	-	32 mm.	-	++	"	"
89	Trace	60 mg. per 100 c.c. (0.9 gm. in 24 hrs.)	20 mg. per 100 c.c.	9.6 gm. in 24 hrs.	36.4 mm.	-	++	"	"
64	Trace	-	31 mg. per 100 c.c.	-	28 mm.	-	++	Pneumonia	
83	Trace	-	11 mg. per 100 c.c.	-	38 mm.	-	++	Typhoid fever	
65	Trace	-	31 mg. per 100 c.c.	-	24 mm.	-	++	Acute alcoholism (coma)	Alcoholic coma.
79	Trace	-	-	-	30 mm.	-	+++	Postoperative vomiting	Severe vomiting following ether anesthesia.
74	Trace	-	-	-	32 mm.	-	+++	Intestinal obstruction	Intestinal obstruction from adhesions with persistent vomiting.
81	Trace	100 mg. per 100 c.c. (0.4 gm. in 24 hrs.)	26 mg. per 100 c.c.	5.8 gm. in 24 hrs.	28 mm.	-	++	Postoperative vomiting	Persistent vomiting following ether anesthesia.
98	4 mg. per 100 c.c.	-	16 mg. per 100 c.c.	-	24 mm.	-	+++	General peritonitis	Autopsy showed extensive cloudy swelling of all organs and marked fatty degeneration of liver.
100	7 mg. per 100 c.c.	330 mg. per 100 c.c. (1.55 gm. in 24 hrs.)	27 mg. per 100 c.c.	6 gm. in 24 hrs.	29 mm.	-	+++	Persistent vomiting of unknown cause	Chronic (3 weeks) periodic attacks of vomiting. Began following laparotomy for pelvic disease and cholecystitis.



TABLE IV.

## NEPHRITIS.

SERIAL NUMBER	BLOOD NH <sub>3</sub> (MG. PER 100 C.C.)	URINE NH <sub>3</sub> (MG. PER 100 C.C. AND OUTPUT IN GM. IN 24 HRS.)	BLOOD UREA (MG. PER 100 C.C.)	URINE UREA (24 HR. OUTPUT IN GM.)	CO <sub>2</sub> TENSION OF ALVEOLAR AIR, IN MM. OF HG.	BICARBONATE TEST, (SEL- LARD'S TEST.)	ACETONE AND DIACETIC ACID IN URINE	DIAGNOSIS	REMARKS
13	6 mg. per 100 c.c.	50 mg. per 100 c.c. (0.52 gm. in 24 hrs.)	140 mg. per 100 c.c.	3.3 gm. in 24 hrs.	30 mm. of Hg.	-	None	Chronic diffuse nephritis (hypertension)	Complicated by dilatation of the heart. Marked chronic passive congestion of liver. (Autopsy.)
17	Trace	-	131 mg.	4 gm. in 24 hrs.	24 mm.	-	None	Chronic diffuse nephritis	Uremia. (Autopsy.)
18	Trace	-	120 mg. per 100 c.c.	2.4 gm. in 24 hrs.	35 mm.	-	None	Chronic diffuse nephritis (hypertension)	
22	4 mg. per 100 c.c.	100 mg. per 100 c.c. (0.8 gm. in 24 hrs.)	20 mg. per 100 c.c.	10.5 gm. in 24 hrs.	32 mm.	-	+	Subacute diffuse nephritis (Cheyne-Stokes type)	General anasarca—dilatation of heart. Marked chronic passive congestion of liver. (Autopsy.)
25	3 mg. per 100 c.c.	-	66 mg. per 100 c.c.	7.2 gm. in 24 hrs.	39 mm.	-	None	Chronic diffuse nephritis. (Hypertension)	
26	1.3 mg. per 100 c.c.	-	19.7 mg. per 100 c.c.	-	28 mm.	-	None	Acute diffuse nephritis	General anasarca—dilatation of heart. Chronic passive congestion. Cheyne-Stokes respiration. Recovery.
28	Trace	40 mg. per 100 c.c. (0.4 gm. in 24 hrs.)	38 mg. per 100 c.c.	9 gm. in 24 hrs.	46 mm.	-	None	Chronic diffuse nephritis	
36	Trace	-	95 mg. per 100 c.c.	4.2 gm. in 24 hrs.	40 mm.	-	None	Chronic diffuse nephritis	
37	Trace	44 mg. per 100 c.c. (0.5 in 24 hrs.)	58 mg. per 100 c.c.	6 gm. in 24 hrs.	38 mm.	-	None	Chronic diffuse nephritis	Marked dyspnea. Chronic passive congestion marked. Death. (Autopsy.)
39	Trace	-	253 mg. per 100 c.c.	-	32 mm.	-	None	Chronic diffuse nephritis	Uremia. Death. Anasarca.
42	Trace	-	40 mg. per 100 c.c.	10 gm. in 24 hrs.	49 mm.	-	None	"	
44	1.2 mg. per 100 c.c.	-	25 mg. per 100 c.c.	-	47 mm.	-	None	"	
47	Trace	90 mg. per 100 c.c. (0.6 gm. in 24 hrs.)	110 mg. per 100 c.c.	8.4 gm. in 24 hrs.	34 mm.	-	+	"	Dyspnea marked. Marked chronic passive congestion of liver.
51	1 mg. per 100 c.c.	-	236 mg. per 100 c.c.	-	15.5 mm.	-	None	Chronic diffuse nephritis	Uremia. (Autopsy.)
57	Trace	-	34 mg. per 100 c.c.	9.2 gm. in 24 hrs.	42 mm.	-	None	"	
68	Trace	48 mg. per 100 c.c. (0.8 gm. in 24 hrs.)	80 mg. per 100 c.c.	-	-	-	None	"	
80	9 mg. per 100 c.c.	93 mg. per 100 c.c. (0.75 gm. in 24 hrs.)	128 mg. per 100 c.c.	2.8 gm. in 24 hrs.	28 mm.	-	None	"	Dyspnea marked. Chronic passive congestion marked. Death. Uremic coma. (Autopsy.)
82	Trace	60 mg. per 100 c.c. (0.5 gm. in 24 hrs.)	328 mg. per 100 c.c.	4.8 gm. in 24 hrs.	34.2 mm.	-	None	"	Cheyne-Stokes respiration. Uremia. (Autopsy.)
86	Trace	-	71 mg. per 100 c.c.	9.6 gm. in 24 hrs.	-	-	None	"	



TABLE V.  
A CASE OF PERSISTENT PERIODIC VOMITING OF UNKNOWN ETIOLOGY.

SERIAL NUMBER	BLOOD NH <sub>3</sub> (MG. PER 100 C.C.)	URINE NH <sub>3</sub> (MG. PER 100 C.C. AND OUTPUT IN GMS. PER 24 HRS.)	BLOOD UREA (MG. PER 100 C.C.)	URINE UREA OUTPUT IN GM. PER 24 HRS.	CO <sub>2</sub> TENSION OF ALVEOLAR AIR (MM. OF HG.)	BICARBONATE TEST. (SEL- LARD'S TEST.)	II-ION CONCENTRATION OF BLOOD	ACETONE AND DIACETIC ACID IN URINE	REMARKS
4 10/26/16	23 mg. per 100 c.c.	80 mg. per 100 c.c. or 0.9 gm. per 24 hrs.	36 mg. per 100 c.c.	13.6 gm. in 24 hrs.	45 mm.	Negative	pH 8.4 (Marriott's Method)	None	Persistent (4 weeks) periodic vomit- ing attacks in a chronic alcoholic. Patient toxic and very drowsy, but irritable and querulous when disturbed. Complains of weak- ness in left leg.
4 11/3/16	17 mg. per 100 c.c.	-	-	-	43 mm.	"		"	
4 11/22/16	12 mg. per 100 c.c.	-	-	-	45 mm.			"	
4 12/3/16	8 mg. per 100 c.c.	-	-	-	46 mm.				No vomiting. Nausea only.
4 12/14/16	Trace	40 mg. per 100 c.c. (0.4 gm. per 24 hrs.)	-	-	45 mm.			"	Vomiting and toxemia have disap- peared. No nausea.
4 12/28/16	3 mg. per 100 c.c.	-	-	-				"	Specimen taken following a recurrence of nausea and vomiting.
4 1/2/17	Trace	-	-	-				"	

TABLE VI.  
TOXEMIAS OF PREGNANCY.

SERIAL NUMBER	BLOOD NH <sub>3</sub> (MG. PER 100 C.C.)	URINE NH <sub>3</sub> (MG. PER 100 C.C. AND OUTPUT IN GM. PER 24 HRS.)	BLOOD UREA (MG. PER 100 C.C.)	URINE UREA (OUTPUT IN GM. PER 24 HRS.)	CO <sub>2</sub> TENSION OF ALVEOLAR AIR. (MM. OF HG.)	BICARBONATE TEST. (SEL- LARD'S TEST.)	ACETONE AND DIACETIC ACID IN URINE	DIAGNOSIS	REMARKS
77	Trace	-	68 mg. per 100 c.c.	-	-	-	+	Nephritic toxemia of pregnancy	Sudden onset with convulsions to- ward latter end of pregnancy. Albumin blood and casts in urine.
105	10.2 mg. per 100 c.c.	-	25 mg. per 100 c.c.	-	-	-	++	Eclampsia	Sudden onset with convulsions to- ward termination of preg- nancy. Albumin and casts in urine. Autopsy showed typi- cal hepatic focal necroses of eclampsia.
99	7 mg. per 100 c.c.	-	27 mg. per 100 c.c.	-	38 mm.	Negative	None	Eclampsia (?)	Postpartum convulsions. Recovery.
91	3.4 mg. per 100 c.c.	110 mg. per 100 c.c. (1.5 gm. per 24 hrs.)	20 mg. per 100 c.c.	8.9 gm. per 24 hrs.	28 mm.	-	++	Vomiting of pregnancy	Persistent vomiting early in preg- nancy. Recovery.

## CONCLUSIONS.

1. Increases of blood ammonia of above 1 mg. per 100 c.c. are occasionally met with in various diseases.
2. Such increases can not be explained by acidosis in all cases.
3. The fact that nine cases showing marked increases (over 3 mg. per 100 c.c.), which have come to autopsy, have shown definite hepatic lesions would suggest some connection between increased blood ammonia and functional insufficiency of the liver.
4. The fact that of ten chronic and subacute cases of inflammatory disease of the liver studied, only one has shown an increase of blood ammonia would indicate acute functional insufficiency of the liver cells rather than acute or chronic inflammatory changes in the liver parenchyma as the source of any blood ammonia increase.

## BIBLIOGRAPHY.

- <sup>1</sup>McNeil and Levy: Jour. Lab. and Clin. Med., 1917, ii, 509.
- <sup>2</sup>Hahn, Massen, Nencki and Pawlow: Arch. f. exper. Path. u. Pharmacol., 1893. xxxii, 161.
- <sup>3</sup>Fischler: Deutsch. Arch. f. klin. Med., 1911, civ, 300.
- <sup>4</sup>Williams: Text Book on Obstetrics, 1916.
- <sup>5</sup>Losee and Van Slyke: Am. Jour. Med. Sc., 1916, cliii, 94.
- <sup>6</sup>Folin: Ztschr. f. physiol. Chem., 1902, xxxvii, 161.
- <sup>7</sup>Medwedew: Ztschr. f. physiol. Chem., 1911, lxii, 410.
- <sup>8</sup>Rhode: Jour. Biol. Chem., 1915, xxi, 325.
- <sup>9</sup>Gettler and Baker: Jour. Biol. Chem., 1916, xxv, 211.
- <sup>10</sup>Bennett: Jour. Biol. Chem., 1917, xxix, 459.
- <sup>11</sup>Folin and Denis: Jour. Biol. Chem., 1912, xi, 161.
- <sup>12</sup>Fridericia: Berl. klin. Wchnschr., 1914, li, 1268.
- <sup>13</sup>Sellards: Bull. Johns Hopkins Hosp., 1914, xxv, 141.
- <sup>14</sup>Fiske and Karsner: Ability of the Liver to Absorb Ammonia, Jour. Biol. Chem., 1913, xvi, 399.
- <sup>15</sup>Fiske and Summer: Urea Formation Outside of the Liver, Jour. Biol. Chem., 1914, xviii, 285.
- <sup>16</sup>Fiske and Karsner: Effect of Destructive Lesions of the Liver on Ammonia of the Blood, Jour. Biol. Chem., 1914, xviii, 381.

# INFECTIOUS MENINGITIS—A STUDY OF 27 CASES IN 586 AUTOPSIES\*

BY STUART GRAVES, M.D., LOUISVILLE, KY.

OF 586 autopsies done in the pathologic laboratory of the Medical Department of the University of Louisville and of the Louisville City Hospital between September 1, 1914, and April 1, 1917, 27 revealed leptomeningitis, a percentage of 4.6. From a study of this material it is my purpose to present some statistics and conclusions of interest and value particularly to the clinician. It must be borne in mind that some of these subjects were coroner's cases which had been sent into the laboratory for autopsy and had not been on the wards at all; some were practically moribund when admitted to the wards; and some, it must be confessed, did not come to autopsy with as complete and accurate records as could be desired. The cases examined were briefly as follows:

## ABBREVIATED PROTOCOLS OF THE CASES.

(1) A-14-3. Adult, male, white, age unknown. Coroner's case.

*Excerpt from chart:* Admitted Sept. 12; provisional diagnosis, septic meningitis. Died that afternoon. Temperature 99.1°; pulse 126; respiration 28. Patient irrational on admission. Six days before, while working in a stable, had become irrational. Three days later condition became so aggravated that he was dismissed from work. Gradually developed coma, in which condition he was brought to hospital with psychomotor unrest, retraction of eyes, contracted pupils, positive Kernig. Lumbar puncture showed bacillus mucosus capsulatus.

*Autopsy findings:* Piarachnoid of cerebrum, cerebellum and cord covered with a thick, yellow, mucoid exudate. Smears and cultures showed Gram-negative, encapsulated bacilli, gas, acidified milk and thick, mucoid growth on agar. Bacteriologic diagnosis, bacillus mucosus capsulatus. Lungs and middle ears negative.

(2) A-14-11. Negro, female, 8 months old.

*Excerpt from chart:* Admitted Sept. 24; died Oct. 4. Clinical diagnosis, tuberculous meningitis. Uncle living in house during first four months of child's life had tuberculosis. Last four months patient had cough, spasms with vomiting and later crepitant and moist rales over right apex. Spinal fluid: No organisms found, moderate increase in lymphocytes. Temperature 98.6° to 103.4°; pulse 130 to 154; respirations 26 to 64.

*Autopsy findings:* General miliary tuberculosis of lungs, liver, spleen, kidneys, ileum and meninges of brain and cord. Grayish exudate along left middle cerebral vessels, beginning over right Sylvian fissure and extending chiefly over base of cerebrum around circle of Willis. Scattered miliary tubercles also found. Cord not removed.

(3) A-14-16. Negro girl 16 years old. Coroner's case.

*Excerpt from chart:* Admitted Oct. 12; died Oct. 16. No history obtainable because of condition of patient. No neck rigidity present afternoon before death. Spinal fluid showed 723 polymorphonuclear leucocytes per cubic millimeter; coagulated in heating for globulin test. No Kernig obtainable. White count, 6,200; polymorphonuclear leucocytes, 66%. Temperature 98.4° to 101°; pulse 92; respirations 28.

*Autopsy findings:* Pulmonary tuberculosis. Miliary tuberculosis of spleen, liver and kidneys. Piarachnoid grayish and slightly thickened along middle cerebral arteries, but no frank exudate. Similar condition in upper portion of cord. Sections showed tuberculous meningitis of cerebrum and cord.

(4) A-14-27. White, male, 23 years old. Coroner's case.

*Excerpt from chart:* Patient had been thrown from motorcycle on Sept. 17, 1914,

\*From the Pathologic Laboratory of the Medical Department of the University of Louisville and of the Louisville City Hospital.

Read before the Jefferson County Medical Society, May 21, 1917.

and sustained fracture of frontal bone. X-ray showed fracture of left frontal bone extending from outer angle of left eye upward and across median line. Ran irregular temperature from 97.4° to 100.4°. Discharged Oct. 15 with normal temperature, pulse and respirations. Readmitted Oct. 23 and trephined Oct. 26. Died that afternoon. No symptoms recorded. Temperature 98° to 104°; pulse 120.2; respirations 22. Lumbar puncture day of admission showed 2070 cells per cubic millimeter; 98% polymorphonuclear leucocytes. Lumbar puncture two days later showed 1330 cells per cubic millimeter; 83% polymorphonuclear leucocytes. Smears and cultures showed pneumococci.

*Autopsy findings:* Entire surface of piaarachnoid of brain and cord covered with a grayish white exudate. Sections showed acute leptomeningitis.

(5) A-14-42. Negro, female, adult, age 59 years.

*Excerpt from chart:* Admitted Nov. 3; died Nov. 6. Complained of pain in head, neck, chest and back. No antemortem diagnosis, lumbar puncture, physical examination or blood count recorded. Temperature subnormal until last day when it rose to 103°; pulse 78; respirations 24.

*Autopsy findings:* On right side of longitudinal fissure piaarachnoid opaque and yellow, most marked along course of vessels. Similar condition along middle cerebral arteries, more marked over base of brain. Smears from exudate showed pus cells and Gram-positive, lancet-shaped diplococci, morphologically pneumococci.

(6) A-14-55. Negro, female, adult.

*Excerpt from chart:* Admitted Nov. 25; died at 3 P.M. Provisional diagnosis: Cerebral hemorrhage.

*Autopsy findings:* Lobular pneumonia, meningitis. Small localized areas of grayish exudate over surface of brain, most marked along margin of cerebellum. Cord grossly negative. Smears from cerebellum showed Gram-positive, encapsulated diplococci, morphologically pneumococci.

(7) A-14-73. Coroner's case outside. Negro, female, adult. No history.

*Autopsy findings:* Entire surface of piaarachnoid of cerebrum, cerebellum, and cord covered with thick, yellow, purulent material, especially along vessels. Middle ears negative. Smears showed many pus cells and Gram-negative, biscuit-shaped, intra- and extracellular diplococci, morphologically meningococci.

(8) A-15-20. Negro, male, adult.

*Excerpt from chart:* Patient admitted Jan. 28, at noon; died at 9 P.M. Admitted unconscious. No history or physical examination on chart. Temperature 101.6° (axillary); pulse 140; respirations 48.

*Autopsy findings:* Lobular pneumonia. Along vessels of pia mater of the dorsum of the brain were seen yellowish, opaque stripes. Over frontal lobes there was a thick, dense, opaque, yellowish material beneath pia entirely concealing the vessels and brain tissue. This thick, dense, opaque, yellowish material found beneath pia in region of pons, medulla, and under surface of temporal lobes. Beneath pia of spinal cord were seen yellowish areas varying from 1 to 4 mm. in diameter, scattered throughout its entire length. Smears showed pus cells and Gram-positive diplococci, encapsulated, morphologically pneumococci.

(9) A-15-33. Negro, male, adult.

*Excerpt from chart:* Admitted March 6; died March 9. Patient delirious; no history obtainable. Neck stiff. Kernig positive. Spinal puncture showed white blood cells, 186; polymorphonuclear leucocytes, 68%; lymphocytes, 32% (Blood?). No tubercle bacilli found. Blood count, March 6; W. B. C., 10,600; polymorphonuclear leucocytes, 80%; R. B. C., 2,500,000. Temperature 100° to 102.4°; pulse extremely irregular, 60 to 150 with daily change; respirations 20 to 40.

*Autopsy findings:* About poles of temporal lobes, pons, and optic chiasm piaarachnoid showed grayish yellow, thick exudate along vessels. Cord showed occasional yellowish gray, rounded nodules 1 mm. in diameter. Smears from meninges of brain and cord showed numerous lymphocytes and tubercle bacilli. Sections showed miliary tuberculosis of lungs, spleen and liver and conglomerate tuberculosis of adrenals. Middle ears, negative.

(10) A-15-45. Negro, male, 58 years old.

*Excerpt from chart:* Admitted March 13; died April 6. History of two attacks of rheumatism, last one six years ago. Clinical signs and symptoms showed consolidation of base of upper lobe on left side. White count just before death showed 1500 leucocytes.

*Autopsy findings:* Vegetative endocarditis. Infarct of left lung. Cerebrospinal meningitis. Piaarachnoid over medulla, pons, and cerebellum and inferior surfaces of frontal and temporal lobes presented a dense, opaque, grayish yellow exudate, 2 to 3 mm.



thick. Similar exudate over entire spinal cord. Smears from meninges showed Gram-positive, encapsulated, lancet-shaped diplococci, intra- and extra-cellular, with cultural characteristics of pneumococci.

(11) A-15-53. Male, negro, 66 years old.

*Excerpt from chart:* Admitted March 19; died April 19. No diagnosis recorded. Temperature ranged between 97 and 100; pulse 72 to 98; respirations 20 to 30.

*Autopsy findings:* Cerebral leptomeningitis. Infarct of right lung. Piaarachnoid showed grayish white streaks along vessels near vertex and over parietal lobe and posterior portion of frontal lobe on either side. Smear from meninges showed Gram-positive, spherical cocci in short chains up to eight units, morphologically streptococci. Middle ear, negative.

(12) A-15-57. Negro, male, adult.

*Excerpt from chart:* Admitted Jan. 21; died April 29. Suprapubic cystotomy done immediately after admission to relieve complete anuria and greatly distended bladder, urethra being occluded with stricture. Patient improved daily until day before death when he had two convulsions and ceased to breath. Temperature irregular, 96.4° to 103°; pulse 90 to 120; respirations 18 to 38.

*Autopsy findings:* Lobar pneumonia. Obliterative pleural and pericardial fibrous adhesions. Acute tubular nephritis. Cerebral leptomeningitis. There was a narrow, dense, grayish white opacity along vessels of both hemispheres on either side of fissure of Rolando, extending 3 to 4 cm., being more prominent near vertex. Smears and cultures from heart's blood and meninges (11 hrs. postmortem) showed morphologic and cultural characteristics of pneumococci.

(13) A-15-58. Negro, male, 45 years old.

*Excerpt from chart:* Admitted April 28 unconscious; died April 30. Provisional diagnosis: Cerebral hemorrhage. White count, 10,000; polymorphonuclear leucocytes, 72%. Lungs: Some mucous rales present; no dullness.

*Autopsy findings:* Lobar pneumonia. Chronic myocarditis. Cerebral leptomeningitis. On either side of vessels in region of Rolandic fissure were grayish white, opaque streaks, more marked in upper portion. Smears showed Gram-positive, lancet-shaped diplococci, morphologically pneumococci.

(14) A-15-78. Female, negro, adult.

*Excerpt from chart:* Admitted May 20 and died almost immediately. Unconscious. Had been sick three weeks with frequent convulsions day and night for three days before admission. Mucous rales present posteriorly over both lungs. Temperature 99°; pulse 120; respirations 32. No Wassermann done.

*Autopsy findings:* Lobular pneumonia. Healing pericarditis. Chronic internal hemorrhagic pachymeningitis. Sections showed also chronic leptomeningitis. Bacteriology of meninges not studied.

(15) A-15-171. White, male, 14 years old.

*Excerpt from chart:* Admitted Sept. 26; died Oct. 24. Discharging ear for six years. Three weeks before admission began to have pain behind left ear. Physical examination revealed tenderness over left mastoid. Mastoidotomy done. Temperature on admission, 101°, continued irregular, 97.4° to 105.2°; pulse 74 to 144, rising to 168 in last three days; respirations 24 to 36 in last three days. No antemortem bacteriology. Clinical diagnosis: Otitis media. Mastoiditis. Secondary meningitis.

*Autopsy findings:* Acute leptomeningitis and otitis media, left side. Left antrum chiseled out and clean. Smears from left middle ear and base of brain showed pus cells and few Gram-positive diplococci, spherical, in short chains up to six units. Organisms did not grow on culture. Diagnosis, morphologically streptococci.

(16) A-15-217. Negro, female, 26 years old.

*Excerpt from chart:* Admitted on Dec. 31; died immediately after admission. Provisional diagnosis, meningitis. Temperature 101.8°; pulse 110; respirations 28. No laboratory findings recorded.

*Autopsy findings:* Acute cerebral leptomeningitis. Narrow, grayish yellow streaks along vessels of cortex in region of Rolandic fissure. These streaks faded out before reaching base of brain. Smears showed Gram-negative, extracellular, biscuit-shaped diplococci.

(17) A-16-11. Negro, female, 48 years old.

*Excerpt from chart:* Admitted Dec. 31; died Jan. 8. Final diagnosis: Lobar pneumonia. Physical examination showed slight jaundice. Temperature day before death, 103°; pulse, 110; respirations, 42.

*Autopsy findings:* Acute endocarditis. Lobular pneumonia with sections showing



acute arteritis. Healing leptomeningitis. Smears and cultures from heart's blood, valvular vegetations along tricuspid valve and from meninges all showed hemolytic streptococci. Middle ears negative. In piaarachnoid and convolutions about cortex of cerebrum were a few pinhead, grayish white areas. Pyorrhea alveolaris marked.

(18) A-16-115. Negro, female, 8 years old.

*Excerpt from chart:* Admitted May 21; died next day. Diagnosis: Tuberculous meningitis. Child had been sick since Thanksgiving. Began vomiting all food taken. Had had high temperature since beginning of trouble. Brought in in dying condition. Emaciated and anemic. Few rales in both apices; bases dull. Legs spastic. Kernig present. In opisthotonos and unconscious condition before death. At midnight convulsions every few minutes. Temperature (axillary), 104.6°; pulse 144; respirations 56.

*Autopsy findings:* Miliary tuberculosis of lungs, spleen, stomach, pancreas, liver, kidneys, cerebrum, cerebellum, and cord. Piaarachnoid over base of cerebrum showed increase in clear watery fluid. Grayish yellow exudate along vessels near Sylvian fissure and over base. Similar exudate over cerebellum with numerous scattered, pinhead areas. Left caudate nucleus showed marked softening in area 2 cm. in diameter which extends to anterior limb of internal capsule. This area was pinkish yellow, soft and mushy. Sections showed tuberculosis. Smears showed tubercle bacilli.

(19) A-16-117. Negro, male, 35 years old.

*Excerpt from chart:* Admitted May 26; died May 26. Diagnosis: Edema and congestion of lungs. Illness began three days before admission with chill and pain in back of head. This was followed by repeated convulsions. Physical examination showed moist rales over both lungs. Leucocytic count, 9,400; polymorphonuclear leucocytes, 74%. Urine showed albumin. Temperature 105.7°; pulse 140; respirations 40.

*Autopsy findings:* Meninges of medulla, pons and base of brain showed grayish yellow, opaque streaks along vessels. Lateral, third and fourth ventricles filled with thin, grayish yellow, purulent material. Middle ears negative. Smears from meninges showed Gram-negative, intra- and extra-cellular, biscuit-shaped diplococci. Smears from fluid in lateral ventricles showed same. Microscopical sections showed lobular pneumonia, infarct of spleen, beginning glomerular nephritis and regeneration of adrenal cortex with numerous mitotic figures.

(20) A-16-122. Negro, male, 32 years old.

*Excerpt from chart:* Admitted and died on June 4. Patient had been struck on head by automobile three days before. Morning of admission had become delirious and violent.

*Autopsy findings:* Fracture of base of skull involving left petrous portion of left temporal bone. Acute leptomeningitis. Suppurative otitis media. Smears and cultures of meninges and middle ear showed pneumococci. Temperature (axillary), 103.8°; pulse 86; respirations 36.

(21) A-16-138. Coroner's case outside. Negro, female, adult. No history.

*Autopsy findings:* Generalized tuberculosis. Tuberculous leptomeningitis. Tuberculosis of choroid plexus. Choroid plexus on either side showed grayish white, multiple, pinhead areas; on section grayish yellow and soft. Sections showed tuberculosis. Smear from meninges showed tubercle bacilli.

(22) A-16-139. Coroner's case outside. No history. Male negro, 24 years old. Body restricted.

*Autopsy findings:* Stellate fracture of base of skull. Acute otitis media, right. Sections showing acute cerebral leptomeningitis extending into cerebral cortex. Smears and cultures from meninges and middle ear showed pneumococci.

(23) A-16-159. Female, negro, 17 years old.

*Excerpt from chart:* Admitted Aug. 6; died Aug. 8. Final diagnosis, tuberculous meningitis. Physical examination showed slight rigidity of spine. All reflexes absent. No sensation below waist. Spinal fluid yellowish and syrupy (Froin's syndrome). Butyric acid test, positive. Temperature 100° to 107°; pulse 124 to 140; respirations 24 to 42. Urine showed albumin. White count 11,600.

*Autopsy findings:* Body restricted. Sections of brain and cord showed tuberculous meningitis.

(24) A-16-169. White, male, 52 years old.

*Excerpt from chart:* Admitted Aug. 22; died Aug. 30. Patient admitted semiconscious. Complained of ear trouble. Three days before death had general convulsions. Neck rigid. Kernig positive on both sides. Spinal puncture showed pus cells and a few Gram-positive diplococci; cultures negative after nine days. Leucocytic count, 14,600 to 25,900. Urine showed albumin. Mastoid operation done.

*Autopsy findings:* Organizing leptomeningitis of brain and cord. Right middle ear negative; left showed mastoidotomy wound and was filled with blood clot. Bacteriology (1 hour postmortem) showed streptococci and staphylococcus aureus of meninges of cerebrum, cord and left middle ear.

(25) A-16-227. White, male, 52 years old.

*Excerpt from chart:* Admitted Dec. 26; died Dec. 29. Diagnosis, otitis media, cerebrospinal meningitis, pneumococcic. Patient had been admitted to hospital on Nov. 26 and discharged on Dec. 16. Had been admitted for pain and discharge in right ear and paracentesis had been done. Had no temperature or other symptoms when discharged. Re-admitted on Dec. 26 with severe pain in right parietal and temporal regions. He staggered when walked and became delirious. On Dec. 27 he became unconscious. Spinal puncture showed pneumococci. Right side of face paralyzed. Marked tenderness over mastoid. Neck rigid. Kernig positive in both legs. Hyperesthesia marked. Temperature varied from subnormal to 102.6°; pulse 82 to 132, rising before death; respirations mostly 20 to 26, rising to 40 before death.

*Autopsy findings:* Acute purulent otitis media. Mastoiditis. Labyrinthitis. Acute diffuse fibrino-purulent cerebral leptomeningitis. Lobar pneumonia. Beginning central necrosis of liver. Acute glomerular and tubular nephritis. Toxic adrenalitis with regeneration (mitoses numerous in cortex). Smears and cultures from heart's blood, cerebral meninges and right fossa over middle ear showed pneumococci.

(26) A-17-1. Negro, female, 40 years old.

*Excerpt from chart:* Admitted Jan. 5; died Jan. 9. Diagnosis, meningitis. Unconscious when admitted, remained unconscious to death. At times had a muttering delirium. Some exophthalmos. Pupils slightly dilated and did not react to light. Reflexes exaggerated. Babinski, positive. Incontinence of urine and feces. Spinal fluid clear; pressure not increased; apparently no cell count made. Another specimen of spinal fluid showed Wassermann positive +++. Temperature showed daily rise, 99° to 104.6°; pulse 88 to 124; respirations 22 to 44.

*Autopsy findings:* Lobar pneumonia. Gumma of calvarium, meninges and cerebrum. Healing cutaneous syphilides. Syphilitic deformity of nose. Chronic valvular and mural endocarditis. Postmortem Wassermann positive +++.

(27) A-17-11. Negro, female, 31 years old.

*Excerpt from chart:* Admitted Jan. 12; died Jan. 23. Provisional diagnosis, multiple bruises and lacerations of right leg. Final diagnosis, tetanus. Eight days after admission patient complained of stiffness of jaws and slight sore throat. Five thousand units of antitetanic serum given subcutaneously and an equal dose given intravenously. Two days later 5,000 more given intraspinally; the following day 6,000 intravenously and 3,000 subcutaneously. That day had slight convulsion of shoulders and head. Patient semiconscious and unable to sleep. Neck and back arched and stiff. More convulsions with rigidity of neck and spine. Frequent chronic convulsions followed with profuse diaphoresis day before death. During last hours lay in stupor. Temperature rose to 100° day of admission and continued with irregular rises as high as 102.4°. Pulse varied daily between 64 and 100, rising to 166 day before death and 106 on day of death. Respirations mostly 20 to 30, rising to 60 day before death. No laboratory examinations recorded.

*Autopsy findings:* Piarachnoidal space over hemispheres contained a slight excess of clear, watery fluid. Vessels distended. In sulci along left Sylvian fissure piarachnoid contained a milky fluid, more marked along vessels. Left middle ear contained a brownish yellow tenacious fluid. Postmortem bacteriology: Smear from left middle ear showed Gram-positive, discrete, lancet-shaped diplococci, morphologically pneumococci. Smear from left cerebral hemisphere showed many polymorphonuclear leucocytes, some lymphocytes and a few discrete diplococci, morphologically pneumococci. On culture a few Gram-positive diplococci were found, but the growth, in spite of sterile precautions in taking culture, was overgrown with a Gram-positive, irregular diphtheroid. Postmortem diagnoses: Healing otitis media, left. Healing leptomeningitis.

#### INFECTIOUS AGENTS AND ROUTES OF ENTRANCE.

In the treatment of meningitis, as well as in its prognosis, it is of the utmost importance to ascertain its causative factor. In this series the infectious agents were divided as follows:

Pneumococcus, 11 cases, 40.7%;

Tubercle bacillus, 6 cases, 22.2%;

Meningococcus, 3 cases, 11.1% ;

Streptococcus, 3 cases, 11.1% ;

Bacillus mucosus capsulatus, 1 case, 3.7% ;

Staphylococcus aureus and streptococcus combined, 1 case, 3.7% ;

Treponema pallida, 1 case, 3.7% ;

Undetermined, 1 case, 3.7%.

It is important also to consider the probable route of entrance of the infection to the meninges and the associated infectious lesions. As nearly as can be ascertained in these cases those factors are shown in Table I.

TABLE I.

ROUTE OF ENTRANCE	NUMBER	PER CENT	PROBABLE SOURCE
Traumatic	3	11.1	Fracture of frontal bone, 1 Fracture of temporal bone, 1 Fracture of base of skull, 1
Hematogenous	14	51.8	Lungs, 6 Generalized tuberculosis, 5 Acute endocarditis, 2 Syphilis, 1
Direct extension	7	25.9	Otitis media, 4 Nares (meningococcus), 3
Undetermined (Tuberculosis, body restricted, 1) (Chronic internal hemorrhagic pachymeningitis, 1) (Unknown, 1)	3	11.1	

Statistics as to age, sex and race are of no particular value if they are not figured in proportion to all patients admitted. The youngest patient was eight months old and the oldest sixty-six years. On the charts of many of the patients the exact age was not stated. However, figuring babes 1 to 5 years old, children 5 to 20, and adults more than 20, the statistics are as follows:

## STATISTICS AS TO AGE.

Babes,	1	4%
Children,	4	14%
Adults,	22	82%

## RACE AND COLOR.

	Black	Per cent	White	Per cent	Total	Per cent
Male	9	33.3	5	18.4	14	51.7
Female	12	44.4	1	3.7	13	48.1
Total	21	77.7	6	22.1	27	99.8

## INJURY AND REACTION.

The injury was severe, with more or less extensive necrosis and acute inflammatory reaction, in the infections due to bacillus mucosus capsulatus, pneumococcus, meningococcus and streptococcus and staphylococcus aureus; less severe, with more localized tissue destruction and chronic inflammation in infections due to tubercle bacilli and treponema pallida and in chronic internal hemorrhagic pachymeningitis, although in only one case, that of a negro baby, was the tuberculous injury and reaction limited to miliary tubercles in the piaarachnoid.

TABLE II.  
LUMBAR PUNCTURE AND DIAGNOSIS.

CASE NUMBER.	MENINGITIS DIAGNOSED ANTEMORTEM	MENINGITIS DIAGNOSED POSTMORTEM	LUMBAR PUNCTURE DONE ANTEMORTEM
1	Yes	Yes	Yes
2	Yes	Yes	Yes
3	Yes	Yes (Tuberculous)	Yes <sup>1</sup>
4	Yes	Yes	Yes
5	No	Yes	No
6	No (Cerebral hemorrhage)	Yes	No
7	No	Yes	No <sup>2</sup>
8	No	Yes	No
9	Yes	Yes (Tuberculous)	Yes <sup>1</sup>
10	No	Yes	No
11	No	Yes	No
12	No	Yes	No
13	No (Cerebral hemorrhage)	Yes	No
14	No	Yes (Chronic internal hemorrhagic pachymeningitis)	No
15	Yes	Yes	No <sup>3</sup>
16	Yes	Yes	No <sup>3</sup>
17	No	Yes	No
18	Yes	Yes	No <sup>3</sup>
19	No	Yes	No <sup>4</sup>
20	No	Yes	No
21	No	Yes	No <sup>2</sup>
22	No	Yes	No <sup>2</sup>
23	Yes	Yes	Yes
24	Yes	Yes	Yes
25	Yes	Yes	Yes
26	Yes	Yes	Yes <sup>5</sup>
27	No	Yes	No

<sup>1</sup>Cases 3 and 9 were not diagnosed tuberculous meningitis antemortem apparently because all the spinal fluid was collected in one tube in each case. In the first case, the chart states, the spinal fluid contained "723 polymorphonuclear leucocytes per cubic millimeter and coagulated in heating for globulin test;" in the second case, "186 leucocytes per cubic millimeter, polymorphonuclear leucocytes 68%, lymphocytes 32%."

<sup>2</sup>Cases 7, 21, and 22 were not ward cases, but coroner's cases sent in for diagnosis without any clinical history.

<sup>3</sup>In these cases the antemortem diagnosis was obvious from the clinical condition. In the first there was a complicated mastoidotomy. In the second the diagnosis was made in the admission room. In the third marked signs of systemic tuberculosis were present in a negro girl of eight with positive Kernig and opisthotonos.

<sup>4</sup>The infecting organism in each case was proved at autopsy to be meningococcus. Case 7 was an outside coroner's case. Case 16 died immediately after admission. Case 19 was in the ward less than one day.

<sup>5</sup>Wassermann on spinal fluid antemortem and on blood postmortem (3 hrs.) positive +++.

As is well known, the tubercle bacilli, once they gain access to the loose tissues of the meninges, may excite a more or less diffuse and acute inflammatory reaction. As to termination, all inflammatory reactions end in resolution or organization. In only two types of these cases was there evidence of organization, in the luetic and tuberculous. None came to complete resolution, although, as we shall show, at least three might have done so if they had been properly diagnosed and treated. No serious complications of organization were found.

#### THE SIGNS AND SYMPTOMS.

Of the signs and symptoms recorded in these cases the most common and striking include the following: (1) *Fever*. The temperature was elevated in



every case except one in which it was subnormal until the day of death when it rose to 103° F. The highest temperature was in tuberculous meningitis in which it was recorded at 107° F., although it reached 105.7° F. in a meningococcus infection, and 105.2° F. in a streptococcus infection; (2) *headache*; (3) *rigidity of neck and back*; (4) *Kernig*; (5) *contracted or dilated and fixed pupils*; (6) *vomiting*; (7) *motor reaction to cortical irritation*; (8) *delirium*; (9) *coma*; (10) *hyperesthesia*; (11) *pathologic spinal fluid*.

Osler says in his textbook on medicine that convulsions "are less common in simple than in tuberculous meningitis and that they were not present in a single instance in the cases which I have seen in pneumonia, ulcerative endocarditis or septicemia."

In this series convulsions are recorded in one case of lobar pneumonia and pneumococcus septicemia, in two of lobular pneumonia and healing pericarditis, in one case of complicated mastoiditis, in one of complicated otitis media and in only one of tuberculosis. Rigidity of the neck is not always present. In one case of tuberculous meningitis the chart stated positively that it was not present the afternoon before death.

The most important lesson to be drawn from this study concerns lumbar puncture and spinal fluid. Table II illustrates graphically the relation of antemortem and postmortem diagnoses and the presence or absence of an antemortem spinal puncture.

From the preceding table it can be deduced that correct antemortem diagnoses were made in 10 of 27 cases (1, 2, 4, 15, 16, 18, 23, 24, 25, 26) and that in these cases proper lumbar punctures and spinal fluid examinations were made in 8, while in 17 of 27 cases (3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 17, 19, 20, 21, 22, 27) correct antemortem diagnoses were not made and in such cases a lumbar puncture was not made in a single case.

#### CONCLUSIONS.

The conclusions are obvious:

1. Correct antemortem diagnoses were established in 100% of cases in which proper lumbar punctures and spinal fluid examinations were made.
2. Correct antemortem diagnoses were not established in 70% of cases in which proper lumbar punctures and spinal fluid examinations were neglected.
3. In drawing spinal fluid the second portion should always be collected in a separate, clean, dry, sterilized test tube. Any blood in the specimen to be examined renders findings unreliable, wastes time and material and may lead to incorrect conclusions.
4. A grave responsibility rests upon the doctor who neglects to have made a proper lumbar puncture and a correct examination of the spinal fluid in all cases of possible meningitis, because the establishment of an early diagnosis and the employment of specific treatment with Flexner's antimeningococcus serum are likely to save lives in meningococcus meningitis at least. The neglect of such measures, conversely, may result in unwarranted fatalities.

I take pleasure in acknowledging careful routine work in the autopsies during the period of this study by members of the laboratory staff, Drs. A. H. Stein, J. W. Moore, H. E. Fust, H. H. Reeder, H. R. Livesay, and T. R. Maxwell.

## THE PREVENTION OF SIMPLE GOITER IN MAN\*

A SURVEY OF THE INCIDENCE AND TYPES OF THYROID ENLARGEMENTS IN THE SCHOOLGIRLS OF AKRON (OHIO), FROM THE 5TH TO THE 12TH GRADES, INCLUSIVE—THE PLAN OF PREVENTION PROPOSED.

BY DAVID MARINE, M.D., AND O. P. KIMBALL, B.S., CLEVELAND, OHIO.

SIMPLE goiter in animals is probably the easiest of all known diseases to prevent. Simple goiter includes all the thyroid enlargements seen in the lower animals and those thyroid enlargements seen in man, except cases properly classified as exophthalmic goiter. Many cases with simple goiter later develop exophthalmic goiter. In brief, simple goiter includes all those thyroid enlargements formerly classified as endemic, epidemic and sporadic. The periods when it most frequently develops are (1) fetal, (2) adolescent, and (3) during pregnancy. Anatomically a wide range of changes may be present, depending on the species of animal and on the stage (duration) of the disease. In man and fowls one more commonly sees the form characterized by an abundance of colloid material—the so-called “cystic or colloid goiter” of older writers, while in goiter of dogs, sheep, cattle, pigs, fish, etc., the accumulation of colloid material is seen only in the late, regressive or quiescent stages. Again in man the adenomatous form is very common and is exceedingly rare if present at all in the lower animals.

It will not be possible to review all the experimental data on which the assertion, that simple goiter in animals is an easily preventable disease, is based. Certain of the more important facts bearing on the subject will be summarized as an introduction to the discussion of the means proposed to attempt the prevention of simple goiter in man.

1. The developmental stage of all goiters is characterized by an increased blood flow, an increase in the size and number of epithelial cells, a decrease in the stainable colloid of the follicular spaces and a marked absolute decrease in the iodine content. The decrease in iodine precedes the cellular changes.

2. Similar thyroid changes (compensatory hyperplasia) invariably occur in the remaining portion of the gland when a sufficient portion of the entire gland is removed. The amount of gland it is necessary to remove in order to cause compensatory hyperplasia varies somewhat with the species of animal, definitely with the age, the diet, and the presence of iodine.

3. The administration of exceedingly small amounts of any salt of iodine thus far tried in any manner completely protects the remaining thyroid against compensatory hyperplasia, even after the removal of three-fourths of the normal gland in cats, dogs, rabbits and rats, fowls and pigeons. Halsted<sup>1</sup> and Hunnicutt<sup>2</sup> reported a series of partial thyroidectomies in dogs in which they failed to obtain the hypertrophy or hyperplasia of the remaining portion and, therefore, con-

\*From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, Cleveland, Ohio.

Aided by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.



cluded that Halsted's earlier and justly classic experiments<sup>3</sup> on the production of compensatory hyperplasia by partial removal were not due to thyroid removal, but to something else, possibly infection. Their failure to obtain compensatory hyperplasia in the second series was really due to the presence of available iodine either from the absorption of iodine painted on the skin or from contact with other dogs, or from inhalation of volatilized iodine from other dogs carrying iodine, or from other sources in the rooms.

4. If most of the thyroid gland is removed before or in the early stages of pregnancy and rigid steps are taken to exclude available iodine, the pups at birth will have enlarged thyroids, as first shown by Halsted,<sup>3</sup> while if available iodine is present, the pups will have normal thyroids.<sup>4</sup>

5. We have repeatedly found that a milligram of iodine given at weekly intervals is sufficient to prevent thyroid enlargement, although other pups of the same litter, living in the same kennel, and eating the same food, regularly developed goiter.

6. The thyroid gland has an extraordinary affinity for iodine, as can readily be shown by perfusion experiments *in vitro* or by injecting small amounts—5 to 20 mg. KI.—into the circulation.<sup>5, 6</sup> Experimentally then the proof is sufficiently complete to demonstrate the underlying principles of goiter prevention in animals and the ease with which they can be applied. From the practical standpoint, the first instance of preventing goiter on a large scale was accidental and in connection with the sheep raising industry of Michigan. Prior to the discovery of salt deposits around the Great Lakes, the future of the industry seemed hopeless, but with the development of the salt industry and its use by the sheep growers, goiter rapidly decreased. The salt contains appreciable quantities of both bromine and iodine and in places these elements are extracted on a commercial scale. The second instance of goiter prevention on a large scale was in brook trout. Some years ago the development of goiter in artificially raised members of the salmon family became alarming and many plants were abandoned on account of the disease. After considerable work, which led to the conclusion that the disease was simple goiter, we were able to completely prevent the disease in several hatcheries, by the use of very small amounts of tincture of iodine added to the water.<sup>7</sup> Later the attempt was made to substitute whole sea fish for part or all of the diet, which, likewise, proved to be, from the practical point of view, a cheaper and simpler method of complete prevention.<sup>8</sup> Similar preventive work with farm stock is being carried out under our direction in some of the valleys of British Columbia, where goiter was so prevalent that farmers were unable to raise hogs, cattle, horses, and chickens on account of myxedema (cretinism). Similar work in the prevention of goiter in hogs was recently reported by Smith.<sup>9</sup> He was able to completely prevent fetal myxedema by the use of potassium iodide to the mother during pregnancy. He, however, used quantities far in excess of those necessary to prevent goiter and myxedema. In spite of this knowledge of the ease and simplicity of goiter prevention in the lower animals, we know of no instance where the attempt has been made to systematically prevent or control the disease in children in large communities, especially those of the Great Lakes Basin, where goiter is so prevalent. Locally, we have been carrying out preventive treatment for the past six years at the Lakeside Hospital Medical Dispensary and have urged local

physicians to do so in their private practices. A great deal has been accomplished in this way, but as it is a public health matter the most practical and economic method would be to utilize the Public School System and the Board of Health. When the Medical Inspection of Schools is more or less independent of the Board of Health, it would be carried out through the Medical Director of Schools. This year it has been possible to begin such work on a large scale in the city of Akron, through the cooperation of the Superintendent of Schools, the Board of Education, and the County Medical Society.

It was decided for the present to limit the prophylactic work to the girl pupils, since adolescence is the most important goiter developing period and since at this period it occurs about six times more frequently in girls than in boys.

The plan now in operation was arranged from the standpoint of simplicity, practicability, economy, and the possible scientific value of the data obtained. Changes will doubtless be made as the work progresses. First a census of the condition of the thyroid gland was taken of all girls between the 5th and 12th grades inclusive and the findings recorded on individual cards, of which the following is a copy:

No.	Date	
Name	School	
Age	Weight	Physical Development
Grade	Class Standing	
Tonsils-Adenoids		
Thyroid	1	
Simple	2	
Adenomas	3	
Thyroid-tract	4	
Duration		
Remarks		

The thyroid examinations of all pupils were made by a single examiner in order to make the standards used constant and the data obtained uniform. It is planned to take the census each year in the same way.

For the prophylactic treatment we have selected sodium iodide on the grounds of economy and ease of administration. Regarding the amounts that should be given, we have no data except those from animal experimentation. As has been pointed out repeatedly, exceedingly small amounts of iodine are needed. One milligram of iodine given weekly, by mouth, is ample to prevent goiter in dogs. In all our dispensary experiments with children we have used either syrup of hydriodic acid or syrup of ferrous iodide, in 1 c.c. doses, daily for two to three weeks, repeated twice yearly, and have recommended their use to clinicians solely because they were the only U. S. P. preparations sufficiently dilute to offset the tendency to use too large amounts.

We have, therefore, arbitrarily selected to use 2 gm. sodium iodide, given in 0.2 gm. doses each school day, for each pupil in the 5th, 6th, 7th, and 8th grades; and 4 gm. given in 0.4 gm. doses each school day for each pupil in the 9th, 10th, 11th, and 12th grades. These amounts will be given twice annually about the first of May and December, at the schools by the teachers or nurses. Bottles were distributed to the several schools, containing the solutions (0.2 gm. NaI in 5 c.c. H<sub>2</sub>O and 0.4 gm. in 5 c.c. H<sub>2</sub>O) in sufficient amounts to give each

pupil electing to take the prophylactic treatment a total of 50 c.c. A record was made both of those who took the treatment and of those who did not. All pupils will be examined annually and the thyroid conditions recorded. These amounts of sodium iodide provide approximately 1700 (1692) mg. of iodine for each pupil of the 5th, 6th, 7th, and 8th grades and approximately 3400 (3384) mg. for the 9th, 10th, 11th, and 12th grades. When one recalls that 25 to 30 mg. saturates the normal thyroid of 20 to 25 gm. and that the thyroid has an extraordinary affinity for iodine, it seems like a prodigious waste and we believe it is. The amounts used at the start were purposely made excessive to provide for any unknown factors and will probably be materially reduced.

*Analysis of the Thyroid Examinations.*—Three thousand eight hundred and seventy-two girls of the 5th, 6th, 7th, 8th, 9th, 10th, 11th and 12th grades were examined and the general result is given in the following tabulation.

TABLE I.  
CONDITION OF THYROID GLAND.

	NORMAL	SLIGHT ENLARGE- MENT	MODERATE ENLARGE- MENT	MARKED ENLARGE- MENT	ADENOMAS	THYROID- TRACT (PERSISTENT)
Total	1688	1931	246	7	39	594
Per cent	43.59	49.88	6.35	0.18	1.01	13.4

The thyroid glands were examined from the standpoint of *normals*, *slight*, *moderate*, and *marked enlargements*, *adenomas*, *persistent thyroglossal tracts* and the pupils for gross manifestations of *myxedema*, and *exophthalmic goiter*. No obvious case of either myxedema or exophthalmic goiter was found.

Under *normal* we have included all glands (a) which are not visible as a bulging of the skin across the trachea (b) having a barely detectable band of thyroid tissue across the trachea on palpation and (c) absence of well-defined thyroglossal stalk (so-called pyramidal process).

Those cases with enlarged thyroids have been divided into three arbitrary groups (1) *slight*, (2) *moderate* and (3) *marked* enlargement. Under *slight enlargement* we have grouped those cases with (a) visible bulging of the skin over the thyroid isthmus (except in the very stout children) and (b) a widened and thickened isthmial band or mass on palpation. If the isthmus can not be seen or felt, it can be felt by having the child swallow, while the finger or thumb is held against the trachea just below the cricoid cartilage.

Under *moderate enlargement* we have grouped those with gross deformity—bulging of the neck laterally from the enlarged lobes and marked bulging of the skin anteriorly from the enlarged isthmus. In approximately 93 per cent the right lobe was larger than the left, which is about the usual percentage.

Under *marked enlargement* we have grouped those cases with excessive deformity. One thousand six hundred and eighty-eight, or 43.59 per cent, of all pupils examined were classed as normal; 1931, or 49.88 per cent, were classed as slightly enlarged; 246, or 6.35 per cent, were classed as moderately enlarged (none of which had been operated upon); 7, or 0.18 per cent, were classed as markedly enlarged, of which two had been operated upon. This gives as totals 2184, or



56.41 per cent with enlarged thyroids and 1688, or 43.59 per cent, with normal thyroids. In 39 cases, or 1.01 per cent, adenomas, single or multiple, were detected. The smallest was approximately 2 cm. in diameter and the largest about 6 cm. These figures are of little value, since they include only the large superficial and favorably located ones.

The thyroglossal tract when present is very readily detected, either slightly to the right or left of, and rarely in, the midline. Only those which extended to the base of the thyroid cartilage were included. In many it was palpable to the hyoid bone. The very small pyramidal processes ending below the cricoid cartilage were not included. Five hundred ninety-four, or 13.4 per cent, of the cases had well-defined thyroid stalks. Physiologically the presence of thyroid tissue in the line of descent of the embryologic thyroid anlage indicates that the gland had undergone enlargement in intrauterine life, whereas normally the tract undergoes absorption beginning according to His<sup>10</sup> in the second month. The presence of large amounts of thyroid tissue about the foramen cecum—the so-called lingual thyroid—or of large masses between the hyoid bone and thyroid cartilage—so-called infrahyoid thyroids—are of the same significance. Excluding the rare congenital defects in the thyroid anlage, the amount of thyroid tissue in the line of descent of the thyroid gland may be used to determine the degree of normality of the thyroid gland in intrauterine life and as first pointed out by Streckeisen<sup>11</sup> it is an excellent index for determining the extent and degree to which a given district is affected with simple goiter. At Basel he found about 79 per cent of the cases coming to postmortem examination had persistent thyroglossal stalks. If the district is extremely goitrous and the mothers are not

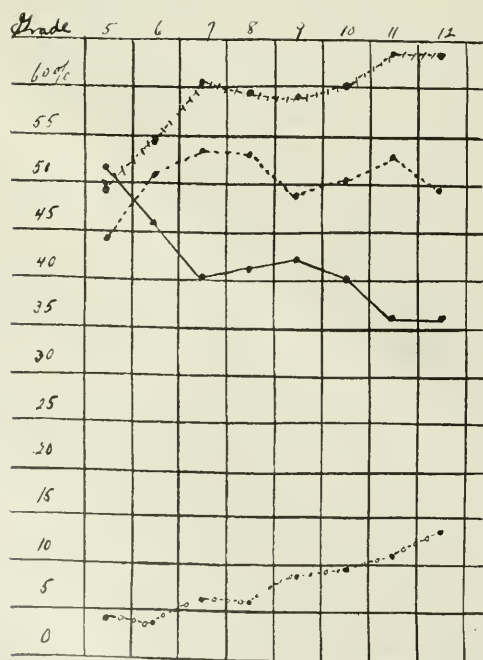


Fig. 1.

— normals; - - - - slight enlargements; -o-o-o-o- moderate enlargements;  
-/-/-/-/- total enlargements.

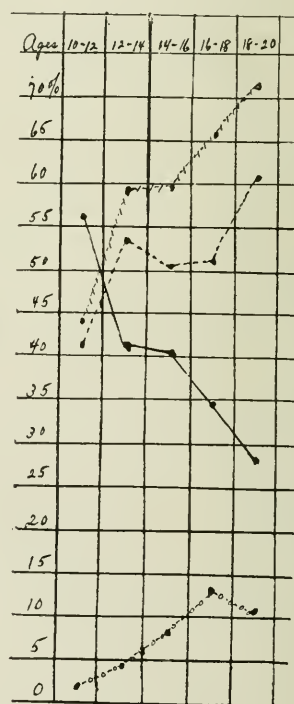


Fig. 2.

fed iodine during pregnancy, practically all children should have large persistent thyroglossal tracts. If the district is nongoitrous (e.g., sea coast regions) very few children will have persistent thyroglossal tracts.

Following the analysis further, the condition of the thyroid in relation to grades is shown in Table II and the accompanying curve chart (see Fig. 1); and in relation to age, in Table III and accompanying curve chart (see Fig. 2).

TABLE II.  
CONDITION OF THYROID ARRANGED BY GRADES.

Grades	5		6		7		8		9		10		11		12	
	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%
Normal	410	51.77	354	45.90	269	40.0	206	40.47	191	42.92	124	40.00	76	36.02	58	36.02
Slightly Enlarged	350	44.20	388	50.33	360	53.49	271	53.24	215	48.31	155	50.00	112	53.08	80	49.70
Moderately Enlarged	31	3.90	29	3.76	43	6.39	31	6.09	38	8.54	30	9.68	23	10.90	21	13.04
Markedly Enlarged	1	0.13			1	0.14	1	0.20	1	0.23	1	0.32			2	1.24
Totals	792	20.45*	771	19.91	673	17.38	509	13.15	445	11.5	310	8.0	211	5.45	161	4.16
Adenomas**	3	0.13	3	0.13	7	0.32	6	0.28	8	0.36	6	0.28	5	0.22	1	0.04

\*Percentage of total pupils examined 3872.

\*\*Adenoma percentage figured from the total enlarged thyroids 2184.

TABLE III.  
CONDITION OF THYROID ARRANGED ACCORDING TO AGES.

Age	10-12		12-14		14-16		16-18		18-20	
	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%
Normal	530	56.08	521	41.32	460	40.35	156	34.44	21	28.77
Slightly Enlarged	394	41.69	680	53.92	578	50.70	235	51.88	44	60.27
Moderately Enlarged	21	2.22	59	4.68	98	8.6	60	13.24	8	10.96
Markedly Enlarged			1	0.08	4	0.35	2	0.44		
Totals	945	24.41*	1261	32.56	1140	29.44	453	11.70	73	1.89
Adenomas	2	0.01**	11	0.52	18	0.84	8	0.39		

\*Percentage of total pupils.

\*\*Percentage of total enlarged thyroids.

The most rapid increase in the number of slight enlargements occurs between the 5th and 8th grades. This corresponds very closely with the rapid increase, between the 10th and 14th years. The average age of the 5th grade pupils was 10 years. Less than 2 per cent of the 5th grade were under 10 years and they were tabulated in the 10 to 12 age group.

The age group 18 to 20 contains less than 2 per cent of the total pupils, and while tabulated for the sake of completeness, the percentages are doubtless higher than the normal average for this age and properly belong to a special group with lower mental activity. The relation of thyroid enlargement to retarded mental development is an important subject, but our available data do not permit of further discussion at present.



## DISCUSSION

The most valuable and accurate data of the incidence of goiter in America can be obtained from examinations of the public school population, because, in the first place it covers the most important ages when goiter develops; secondly, it gives the most complete census; and thirdly, no additional expense or additional effort is necessary. Up to the present time no organized and systematic effort has been made in this country to study the incidence of goiter in the school populations of large communities, even in the Great Lakes Basin—the largest and most densely populated of all goiter districts of North America.

The report by Hall<sup>12</sup> of the examination of 3339 students at the University of Washington is the most extensive available in American literature. Of the 2086 men with the average age of 20 years and 5 months, he found 374, or 17.93 per cent, with enlarged thyroids; 272, or 13.03 per cent, classed as perceptible; 92, or 4.43 per cent, classed as medium; and 10, or 0.48 per cent, classed as large. Of the 1253 women, with the average age of 19 years and 3 months, he found 388, or 30.98 per cent, with enlarged thyroids; 294, or 23.45 per cent, classified as perceptible; 85, or 6.79 per cent, classified as medium; and 9, or 0.7 per cent, classified as large. These figures demonstrate clearly the prevalence of goiter in the northwestern states. The group is too selective and the ages too advanced to give an average incidence percentage, because (a) the greatest incidence occurs during puberty, (b) a certain percentage of enlargements recede below the level of clinical detectability spontaneously and (c) a small percentage would have receded because of iodine feeding.

In the Great Lakes Basin, Olsen<sup>13</sup> reports the examination of 606 women and 193 men, presumably between the ages of 18 and 60, at Chicago. Among the women, he found an average of 17.87 per cent affected and among the men 6.72 per cent. The figures emphasize the frequency of thyroid enlargements, though they are very much lower than would be obtained from a similar number of examinations during the school age, on account of the factors of spontaneous or induced regression of the thyroid enlargements and of migrations from nongoitrous districts, which his figures necessarily include.

In Europe the statistics of Schittenhelm and Weichardt<sup>14</sup> deal with the incidence of goiter in the school populations of certain districts of Bavaria, where goiter is prevalent. Using very liberal standards, they report incidences as high as 77 and 89 per cent of the school population affected.

In the Vosges mountains of eastern France and Alsace, MacAuliffe<sup>15</sup> has recently reported the examination of 2311 children between the ages of 2 and 15 years. He found 288, or 12.5 per cent, affected. A comparison of our data with the data cited above is not possible. We use a much more rigid standard of normal, both clinically and anatomically. Anatomically, the strictly normal gland does not exceed 0.5 gm. thyroid per kilo of body weight, though many European writers, especially those in the Alpine goiter districts, allow as much as 1.0 gm. per kilo. In dogs, the normal thyroid gland does not exceed 0.3 gm. per kilo. Clinically, in the normal gland the isthmus can barely be felt, but the lateral lobes can not be felt.

The question of the production of exophthalmic goiter by the use of iodine may be mentioned briefly. Some Swiss writers, like Oswald<sup>16</sup> take the extreme

view that iodine should never be used in goiter, because of the danger of producing exophthalmic goiter. Pineles<sup>17</sup> and Kocher<sup>18</sup> take the more moderate ground that iodine should be given cautiously to neurotic individuals with goiter. Our experience has led us to the conclusion that the risk of inducing manifestations of exophthalmic goiter from the use of iodine in physiologic doses is exceedingly small, even in those cases with large hyperplastic thyroids; i. e., the kind of thyroid enlargement which would permit of the most rapid formation and excretion of the iodine-containing hormone. The extent to which iodides are used in general medicine and surgery and the rarity of the development of signs of exophthalmic goiter is the best index of the danger. Iodine is usually employed in immensely large doses; 0.2 to 0.4 gm. NaI daily for 2 weeks would offer a great excess over the amounts necessary to saturate even the largest thyroids and probably much smaller amounts would suffice in man, as it has been proved to do in the lower animals.

While the danger of causing symptoms of exophthalmic goiter probably varies with the size and degree of active hyperplasia, all authors agree that the important factor in determining such symptoms lies outside the thyroid, either in the nervous system, or some gland like the adrenal, and antedates any thyroid changes. Klose<sup>19</sup> has reported the production of exophthalmic goiter in nervous fox terrier dogs, by the injection of sodium or potassium iodide in 0.6 gm. doses per kilo. Those experiments were soon discredited by the work of Bordenhewer<sup>20</sup>. No one else has suggested any danger from the use of iodides in the case of nongoitrous individuals, except the well-known acute iodism, which affects a small percentage of people, and, so far as known, is not related to thyroid activity. Cases with definite manifestations of exophthalmic goiter should not be given iodine, although there are cases (or better, stages) of the disease which are distinctly benefited by iodides.

The use of desiccated thyroid has well-known dangers after adolescence—mainly because of the large doses used. Both economically and practically, it would not be suitable for general use, as a prophylactic agent.

#### SUMMARY

In a complete census of the condition of the thyroid gland in the girls from the 5th to 12th grades of the school population of a large community in the Great Lakes goiter district, it was found that 1688, or 43.59 per cent had normal thyroids; 2184, or 56.41 per cent, had enlarged thyroids; and 594, or 13.4 per cent, had well-defined, persistent thyroglossal stalks. The community lies near the southern edge of the goiter district and it is suggested that communities near the lakes would show a higher incidence. The method of prophylaxis proposed is in operation.

#### BIBLIOGRAPHY.

- <sup>1</sup>Halsted, W. S.: Reconsideration of the Question of Experimental Hypertrophy of the Thyroid Gland, and the Effect of Excision of This Organ Upon Other Ductless Glands. *Am. Jour. Med. Sc.*, 1914, cxlviii, 56.
- <sup>2</sup>Hunnicutt: Absence of Hyperplasia of Remainder of Thyroid in Dog After Piecemeal Removal of this Gland. Autotransplantation of Thyroid in Partially Thyroidectomized Animals, *Am. Jour. Med. Sc.*, 1914, cxlviii, 207.
- <sup>3</sup>Halsted, W. S.: Experimental Study of Thyroids of Dogs, *Johns Hopkins Hosp. Report*, 1896, i, 373.
- <sup>4</sup>Marine and Lenhart: Effects of the Administration or the Withholding of Iodin-Con-

- taining Compounds in Normal, Colloid, or Actively Hyperplastic Thyroids of Dogs. Some Experiments on Prenatal Thyroid Hyperplasia in Dogs, etc., *Arch. Int. Med.*, 1909, iv, 253.
- <sup>8</sup>Marine and Feiss: The Absorption of Potassium Iodid by Perfused Thyroid Glands and Some of the Factors Modifying It, *Jour. Pharm. and Exper. Therap.*, 1915, vii, 557.
- <sup>9</sup>Marine and Rogoff: The Absorption of Potassium Iodid by the Thyroid Gland in Vivo Following Its Intravenous Injection in Constant Amounts, *Jour. Pharm. and Exper. Therap.*, 1916, viii, 439.
- <sup>10</sup>Marine and Lenhart: Observations and Experiments on the So-called Thyroid Carcinoma of Brook Trout (*Salvelinus Fontinalis*) and Its Relation to Ordinary Goiter, *Jour. Exper. Med.*, 1910, xii, 311.
- <sup>11</sup>Marine: Further Observations and Experiments on Goiter (So-called Thyroid Carcinoma) in Brook Trout (*Salvelinus Fontinalis*). III Its Prevention and Cure, *Jour. Exper. Med.*, 1914, xix, 70.
- <sup>12</sup>Smith, G. E.: Fetal Athyreosis. A Study of the Iodin Requirements of the Pregnant Sow, *Jour. Biol. Chem.*, 1917, xxix, 215.
- <sup>13</sup>His, W.: Der Tractus Thyreoglossus und Seine Beziehungen zum Zungbein, *Arch. f. Anat. u. Physiol. (Anat. Abtheilug)*, 1891, 26-32.
- <sup>14</sup>Streckeisen: Beiträge zur Morphologie der Schilddrüse, *Virchows Arch. f. path. Anat.*, 1886, xiii, 131, 215.
- <sup>15</sup>Hall, D.: The Prevalence of Goiter in the Northwest, Based on the Examinations of 3339 Students Entering the University of Washington, *Northwest Medicine*, 1914, vi, 189, 371.
- <sup>16</sup>Olsen, E. T.: Goiter, Its Prevalence in Chicago, as Shown by the Examination of 800 Individuals, *Illinois Med. Jour.*, Springfield, 1915, xxvii, 16.
- <sup>17</sup>Schittenhelm, A., and Weichardt, W.: Der Endemische Kropf mit Besonderer Berücksichtigung des Vorkommens in Königreich Bayern J. Sprenger, Berlin, 1912.
- <sup>18</sup>MacAuliffe, L.: Goitre, cretinisme et myxedème dans les Hautes-Vosges, *Bull. de l'Acad. de méd.*, Paris, lxxv, 127.
- <sup>19</sup>Oswald: Die Gefahren der jodbehandlung correspondenz., *Bl. f. Schweizer Aerzte*, 1915, xlv, 641.
- <sup>20</sup>Pineles: Ueber die Empfindlichkeit des Kropfes gegen Jod, *Wien. klin. Wchnschr.*, 1910, xxiii, 353.
- <sup>21</sup>Kocher: Ueber jod Basedow, *Arch. f. klin. Chir.*, 1910, xcii, 1166.
- <sup>22</sup>Klose: Experimentelle Untersuchungen über die Basedow'sche Krankheit, *Arch. f. klin. Chir.*, 1911, xcv, 649.
- <sup>23</sup>Bordenhewer: Erzeugt. Iodeinspritzung Morbus Basedow? *Arch. f. klin. Chir.*, 1912, xcvi, 729.

## TRANSPLANTATION OF THE THYMUS IN RABBITS—RELATION OF THE THYMUS TO SEXUAL MATURITY

BY DAVID MARINE, M.D., AND O. T. MANLEY, M.D., CLEVELAND, OHIO.

THE objects of these experiments were to obtain more definite data on the transplantability of the thymus; and if transplantable in accessible locations, to utilize this means of studying its behavior in relation to sexual maturity and breeding.

The thymus normally undergoes a striking atrophy or involution at puberty. It seems well established by the work of Paton and Goodall, Henderson, Calzolari and others, that removal of the gonads before puberty delays thymus involution; that thymus removal hastens sexual maturity in rabbits; and that animals allowed to breed show earlier thymus involution than those not so used.

In our experiments, we have removed all the main thymus mass (exposing the gland by splitting the sternum to the third rib) except the upper portion of one of the cervical cornua, and have transplanted small (2 to 3 mm.) fragments into the subcutaneous tissue of the abdomen. Contrary to the results of Renton



that transplants in the subcutaneous tissues did not survive, we have found that it can be readily transplanted in this location in sexually immature rabbits. Whether the peritoneal or subperitoneal tissues are still more favorable, as some authors state, we have no data.

As with the spleen, only autotransplants have survived. Immediately after thymectomy, as above described, two autotransplants were placed in the subcutaneous tissue of the abdomen of each of 8 rabbits. Each rabbit was between three and one-half and four months old. Six of these rabbits have been observed for three months, and the transplants examined directly at monthly intervals. Two females were kept with one of the males. Examination at the end of the first month showed that both were pregnant, which is earlier than rabbits usually breed. At the gross examination, the transplants in all three seemed negative, though the enlarging breasts made the examinations unsatisfactory. One transplant area was removed from each and examined histologically. In one female, there was an active transplant, while in the other female and the male the transplants had undergone nearly complete absorption. Of the remaining five, two died before the end of the first month. The remaining three, (two females and one male) had active transplants, the male and one female having large 4 mm. transplants, showing clearly that growth had occurred.

At the beginning of the second month, the female with the large thymus transplants was bred, and at the end of the second month these transplants could not be found, though on account of the lactating breasts the examination was not satisfactory. The male with large transplants at the end of the first month had active transplants, possibly larger than at the first examination. The unbred female also had active transplants.

Again at the examination after three months, the male and female (now over seven months old) kept isolated, still had active transplants. One from each was removed for histologic examination. Microscopically, these are encapsulated vascular masses of compact lymphoid tissue. There is no increase in fibrous tissue about thymus transplants as is usually seen around spleen grafts.

The remaining four (used for breeding) were also examined at the end of three months, and the five remaining transplant areas removed. Definite but small masses of thymus lymphoid cells were found in two.

These experiments, which are preliminary, show that in sexually immature rabbits, fragments of thymus autotransplanted into the subcutaneous tissue of the abdomen after thymectomy may "take," grow, and survive. There is clear though scant evidence in confirmation of other observers' results, that thymus removal hastens sexual maturity. Also, as others have found, utilization of rabbits for breeding hastens involution of the thymus. Our experiments show that this applies to the transplanted thymus as well, and this suggests that a specific nerve influence is not essential for these involutionary changes.

## ON THE COMPLEMENT-FIXATION TEST IN TUBERCULOSIS WITH BESREDKA'S ANTIGEN\*

BY J. BRONFENBRENNER, PH.D., BOSTON, MASS.

REFERRING to the value of auscultation as an aid in early diagnosis of the tuberculosis, Laennec wrote in the preface to his capital work, "I may say that no one who has made himself expert with this method will have occasion to say with Baglivi, 'Oh, how difficult it is to diagnose the disease of the lungs.'"

In spite of the great contribution rendered by Laennec himself, in spite even of the epoch-making discovery of Koch, the early diagnosis of tuberculosis is still a problem to be solved, even as it was one hundred years ago in the days of Laennec.

The difficulties confronting the students of tuberculosis are many: but essentially the problem is difficult because of the extreme pleomorphism of clinical manifestations of tuberculosis and because of peculiar lack of uniformity in the course of this disease in different individuals affected, depending on their susceptibility or individual resistance, which are often subject to temporary fluctuations.

Although methods for the early diagnosis of tuberculosis are still wanted, the wide distribution of this disease is fairly well established. In fact, tuberculosis, not unlike syphilis, must be constantly kept in mind in making a diagnosis, whatever may be the clinical picture of the case.

Unfortunately, the manifestations of syphilis, are no less pleomorphic than those of tuberculosis, and in many instances they may amazingly simulate the clinical picture met with in tuberculosis. In cases where material for bacteriologic diagnosis of tuberculosis is not at hand, Wassermann reaction is practically the only method for the differential diagnosis. However, as the Wassermann test itself is not absolutely specific, and as, on the other hand, it is not always present even in the cases with definite signs of lues, this test does not offer an absolute means of differentiation between the two conditions.

In the quest for a reliable method for a diagnosis of tuberculosis, numerous investigators repeatedly attempted to apply the new methods used in diagnosis of other infectious diseases, but their attempts were crowned with partial success only. The use of agglutination precipitation, miostagmin or epiphanin reactions was found to be of little diagnostic value.

The remarkable usefulness of Wassermann reaction especially stimulated the efforts of numerous investigators along the lines of application of Bordet and Gengou reaction to the diagnosis of tuberculosis. The earlier efforts along this line were not very encouraging. This could be due to several causes. It is possible that (due to the walled-off nature of the lesions and the slow process of the disease in certain cases) there may be none or a very small amount of immune bodies present in the circulation. Moreover, the concentration of circulat-

\*From the Department of Preventive Medicine, Harvard University Medical School.  
Read before Laennec Society of the Johns Hopkins Hospital, Feb. 26, 1917.



ing antibodies is subject to constant and quite marked daily fluctuation in the same patient. Besides, the antibodies in tuberculosis may not be to any great extent of the nature of amboceptor. Again tuberculous amboceptor, as suggested by Davidowitch, may be more thermolabile than most others, and since complement deviation is usually performed with inactivated serum, the amboceptor may be largely<sup>1</sup> destroyed in heating; thus the amount remaining in the serum may not be large enough to be detected even by the delicate method of complement fixation.

In addition to the difficulties due to the apparent peculiarity of the immune processes in tuberculous individuals and resulting variations in concentration of specific amboceptors the lack of proper antigens seems to have been greatly responsible for the irregularity in the results obtained by the different authors in their study of the complement fixation in tuberculosis. The study of the literature on this subject reveals the fact that suspensions of living or dead bacteria were found most reliable of all the different antigens used, but even with such antigens the results obtained were not entirely satisfactory. In 1913, Besredka succeeded in cultivating tubercle bacilli on an entirely new medium. The fact that on this medium the organism showed some hitherto unknown properties induced him to try the antigenic properties of his new cultures for the complement-deviation test. Successful results obtained by Besredka and Manoukhine in their preliminary experiments were fully confirmed in several laboratories. It was found that the complement deviation with Besredka's tuberculin gives a very high (90 to 95 per cent) percentage of positive results in cases of clinical tuberculosis and at the same time the occurrence of the reaction in cases in which tuberculosis could not be detected clinically, was limited to less than 10 per cent.

The antigen of Besredka consists of autoclaved filtered culture of tubercle bacillus grown on a new liquid medium, composed of alkaline broth to which is added egg white and egg yolk.<sup>2</sup>

As this medium can not be made in large quantities on account of its rapid deterioration and has to be made fresh every time, the composition of the antigen made from it may vary in different batches. First of all, the chemical composition of individual eggs, and consequent variations in the amount of alkali necessary for suitable clarification, vary to a marked degree. Besides directly affecting the chemical composition of the antigen, these fluctuations in the medium may also at times enhance or inhibit the growth of bacteria and thus still further influence the respective values of different batches of tuberculin for complement fixation.<sup>3</sup>

Another important source of variation was found in the apparent strain specificity displayed by different tuberculous sera. It is easy to demonstrate

<sup>1</sup>It is known that about 50 per cent of hemolytic amboceptor is destroyed by inactivation.

<sup>2</sup>This medium is made in the following way: Each, white and yolk of an egg are diluted with ten volumes of water and filtered through a hard paper (Chardin). The yolk solution is carefully clarified by the gradual addition of sodium hydroxide. Both solutions are autoclaved and kept separate. Just before using, one mixes 10 volumes of the sterile alkaline veal infusion (prepared without peptone, salt, or glycerin) with two volumes of the sterile egg white solution and one volume of the sterile clarified egg yolk solution. This mixture is placed in sterile tubes and is used without further sterilization. As this medium deteriorates on standing, it should be made fresh each time. Our experience fully confirmed the statement of Besredka about the rapid growth of certain strains of tubercle bacillus on this medium. It is true, not all the strains seem to grow with equal facility, but certain strains, like II-46, II-48 from Dr. Baldwin, H-12, H-29, H-31, from Dr. Theobald Smith, R-3 from Dr. Paul Lewis, and II-389 from Dr. W. H. Park showed very definite growth within the first few days after planting.

that certain tuberculous sera may fix the complement in the presence of certain selected samples of Besredka and other antigens. It is thus, that among 50 cases of diagnosed tuberculosis only in 18 we found fixation with every one of the three samples of Besredka's tuberculin. In 26 cases we obtained fixation with two samples out of three and in two cases the fixation was obtained with one sample of tuberculin only.<sup>4</sup> That the selective fixation with different antigens is due to the strain specificity and not to some technical error is evidenced by the fact that in many instances in which sera were examined repeatedly the results of such examinations invariably confirmed the selective tendency on the part of the sera.<sup>5</sup>

The greatest and most important source of variations in the antigenic value of different batches of Besredka's tuberculin lies, however, in another direction. Already in the beginning of the study it appeared that a number of sera giving a positive Wassermann reaction often showed fixation with Besredka's tuberculin also. As not in all of these cases one could find clinical evidence of tuberculosis, the first explanation for this coincidence of the reactions, naturally was that the sera of syphilitics, having high lipotropic coefficient, fixed the complement also with the tuberculin of Besredka on account of lipoids contained in it. The study of a number of sera giving a positive Wassermann reaction with this point in mind showed, however, that the complement-deviation test with Besredka's antigen is not lipotropic in nature. Whenever present in serums possessing high lipotropic property, it depends on the presence of a separate antibody having its own index different from the lipotropic index in the same serum. Moreover, when the serum deviates the complement in presence of both Besredka's tuberculin and pure lipin antigen, each of the two antibodies can be exhausted from such serum independently of each other. On the other hand, Besredka's tuberculin can be freed of its lipins without losing its property to fix complement in presence of tuberculous sera. The lipins may be extracted by fat solvents, but the easiest method was found to be that of separation of the protein fraction by precipitation.<sup>6</sup>

The presence in the same serum of the property to fix complement with both antigens independently can be reproduced in experimental animals. When present in human beings (as well as in animals), the lipotropic antibody disappears under salvarsan treatment, whereas the tuberculous antibody persists.

The high percentage of negative reactions among clinically nontuberculous (92 per cent), together with the other proofs of the high degree of specificity

<sup>4</sup>This consideration should be given a great deal of attention, as some of the batches of antigen sent to us by Besredka were more anticomplementary than others without being more antigenic; hence it is necessary to vary the amounts of tuberculin used for the test with each batch of antigen.

<sup>5</sup>Among 4000 sera examined in collaboration with J. Rockman and M. J. Schlesinger, with a view of establishing the nature of these variations in the respective fixing power of different samples of Besredka's antigen, the reaction was found positive with one or more samples of this tuberculin in 232 instances. Each of the 232 serums thus selected was reexamined with seven different preparations of tuberculin. We found that in 167 cases (or 72%) out of 232 the fixation was obtained with at least four antigens out of seven used. Out of the remaining 65 cases, in 23 at least two tuberculins fixed the complement, and in 12 cases the crude tuberculin of the New York Board of Health was the only one confirming the results obtained with the tuberculin of Besredka. In 30 cases out of this total of 232, the fixation occurred only with the tuberculins of Besredka.

<sup>6</sup>The existence of strain specificity in tuberculosis may be one of the contributing factors in causing much variation in the results obtained by different investigators in the complement-deviation test for diagnosis of tuberculosis.

<sup>6</sup>Such precipitation of the antigenic fraction of tuberculin also offers the possibility of using a standard number of units of antigen and thus eliminating variations due to the quantitative differences in specific properties of different samples of tuberculin, without increasing the chance of obtaining lipotropic reactions.

of this reaction, seem to indicate that the comparatively frequent simultaneous occurrence of the complement-deviation test with Besredka's antigen and the Wassermann reaction in syphilitics is not due to some technical error.<sup>7</sup> In fact the study of a number of cases from this point of view brings out some very interesting statistics. Thus Jones,<sup>8</sup> subjecting to the Wassermann test 251 unselected cases coming to the public tuberculosis clinic, found that 73 among them had a positive Wassermann. In the series of 346 tuberculous inmates of the Boucicaut Hospital, 19 per cent gave a positive Wassermann test, according to Letulle<sup>9</sup> and of those only 10 individuals (out of 64 reacting) were aware of their syphilitic taint or had definite signs of it. Our own results, as well as these and other similar observations of different authors, which came to our attention since our earlier work was published, suggest that occurrence of the complement fixation with Besredka's antigen in the cases in which Wassermann reaction is also present may be due to the fact that either syphilis, as such, or the antisyphilitic treatment markedly lowers the resistance of the patients so as to make them either more susceptible to new infection with tuberculosis or to render them less resistant against the progress of this disease previously contracted.

Some authors, it is true, did not find any high frequency of occurrence of tuberculosis among syphilitics (as interpreted by the complement-fixation test). However, if one takes into consideration the class of patients upon which the test is performed, this discrepancy can be easily explained. In our series of syphilitics, we dealt with patients of all ages, greatly exposed to infection with tuberculosis in their factory surroundings, and even more so in the unhealthy conditions of their life in the slums. The material of Craig, who found less than 1 per cent of tuberculosis inluetics, for instance, consisted, on the contrary, of a group of young men of military age comparatively free from tuberculosis at the time of their admission to military service and who ever since their admission were placed in exceptionally good hygienic conditions.<sup>10</sup> It seems, therefore, that for a nonselected group of patients (as represented, for instance, by the admissions to the general hospital or dispensary) the simultaneous occurrence of fixation with tuberculin and lipoid is quite frequent and is due to the simultaneous coexistence of two diseases. Such a conclusion is apparently borne out by clinical observations of such men as Fournier, C. F. Marshall, Douty, F. H. Andrews, Sir Jonathan Hutchinson and others.<sup>11</sup>

As for the percentage of the occurrence of the reaction in different stages of tuberculosis when a purified antigen of Besredka is used, we wish to present these approximate figures: First stage, 84 per cent; second stage, 94 per cent; third stage, 15.3 per cent; clinically nontuberculous (controls), nonsyphilitics, 5 per cent.

The question of surprisingly low percentage of positive results in far advanced cases was especially investigated. In addition to the Besredka antigen we examined a large group of such cases (from Leech Farm, Pittsburgh), with the antigens of Craig, Corper and Calmette, and more recently also with antigen

<sup>7</sup>In this connection see results of Fraser, who, using various antigens not containing lipoids came to the conclusion that sera of syphilitics often deviate complement in presence of tuberculosis antigens (bacterial emulsions). *Ztschr. f. Immunitätsforschung*, Orig. 1913, xx, 291.

<sup>8</sup>Jones: *Med. Record*, Sept. 2, 1916.

<sup>9</sup>Letulle: *Bull. de l'Acad. de méd.*, Paris, lxxviii, No. 16, 589.



of Miller. Although it was possible to observe a slight variation in the results obtained with respective antigens, in general they reacted no better than that of Besredka.

As for the reason for this failure of advanced cases to give fixation, there can be at least two offered tentatively: one is that the resistance of the patient has been exhausted, there is no new antibody formation; and the other, that the circulating antibody is taken up as formed by the combination with antigen which may greatly increase during the last stages of the disease.

While the results of experiments on animals in the case of tuberculosis have but a relative value, it might be stated that the general tendency of these results, however, also suggest that the concentration of circulating antibody depends on the degree of resistance of the individual animal. Thus, rabbits seem to develop antibody and show the fixation of complement if infected with the human strains of tubercle bacillus, but usually fail to fix the complement when infected with bovine. In guinea pigs the results are even more convincing. The animals give complement-fixation as early as the fourth or fifth day after infection, but uniformly fail to fix complement during the fourth to sixth week of the disease.

Before closing I would like to give in brief the procedure followed in performing the test.<sup>11</sup>

Fresh serum is used, thus obviating the danger of destruction of antibodies due to heating. The human serum is titrated for the amount of complement present. Such titration is performed with washed human red blood cells which have been previously sensitized with one unit of amboceptor. (The antihuman hemolytic system is used to avoid the uncontrollable factor due to the presence in human serum of varying amounts of natural antisheep amboceptor.) At the same time guinea pig serum is titrated for its complement content, using human erythrocytes sensitized with the same amount of amboceptor. In setting up the test, one adds a sufficient amount of guinea pig serum to bring up the amount of complement already present in the human serum to two units. The antigen is then added and the tubes are incubated. At the end of the incubation, erythrocytes sensitized with one unit of amboceptor are added and after another period of incubation the progress of hemolysis is noted. We found this method to be very sensitive, its delicacy being entirely due to the minute amount of complement available for fixation and the absolute control of hemolytic system. Another advantage is in the fact that by using active serum we do not miss weak cases, where heating would have destroyed the antibody. As for the antigen to be used in the test, although we found Besredka's antigen to give the best results, we must admit, that the preparation of antigen as suggested by Miller and Zinsser is much simpler, and if the results obtained with it are as good as those obtained with Besredka's tuberculin, it seems that such antigen might be more practical for the use in the test.

It is evident that a successful application of the complement-fixation re-

<sup>10</sup>As a characteristic example of another extremity showing the importance of ascertaining the social status of the patients on whom such tests are performed with the view of drawing conclusions as to frequency and interdependence of these two diseases I wish to quote from Dr. N. B. Potter's article the results of Dr. Tedeschi who found in his ten years' service as a physician in a prison that 70 per cent of the cases of pulmonary tuberculosis had developed upon a luetic soil. Tedeschi: *Studium*, Napoli, 1910, iii, 343, 377.—Potter: *Am. Jour. Med. Sc.*, Dec., 1916, vol. 6, p. 823.

<sup>11</sup>Oxford System of Syphilis, 1914, iii, 197.

<sup>12</sup>A detailed discussion of the technic is given in *American Journal of Syphilis*, 1917, i, 2, p. 406.

ction for the diagnosis of early tuberculosis is already at hand. There is very little doubt that fixation indicates active tuberculosis, especially if the reaction remains positive when repeated at intervals. In arrested or cured cases the reaction eventually becomes negative. The value of the negative outcome of the test is only relative (as it is also in case of the Wassermann reaction), however, inasmuch as in advanced cases it is negative as well. In such cases the absence of circulating antibody may indicate the failing of resistance.

## A CASE OF CONGENITAL CYSTIC KIDNEY IN WHICH A TUBERCULOUS PROCESS WAS SUPERIMPOSED\*

BY PAUL G. WOOLLEY, M.D., CINCINNATI, OHIO.

H. G., Hospital No. B-876, a married negress, 41 years old, was admitted to the Cincinnati General Hospital on February 5, 1917, complaining of shooting pains in the region of the right kidney.

FAMILY HISTORY.—Negative.

PAST HISTORY.—She had had measles and whooping cough. At the age of 18, she had typhoid. In November, 1915, she was operated upon for a renal tumor. Menstruation commenced at the age of 13½, and has been somewhat irregular and the flow slight. She has been married for 13 years. Her husband is living and well and they have three children. There have been no miscarriages.

About a year before the operation of November, 1915, she had the same sort of pain of which she complains now; i.e., shooting pain radiating down to the bladder and around to the back. She was unable to do much work because of lassitude. She had some little pain after urinating and at times she passed blood. The operation disclosed a right kidney studded with cysts which varied in size from a pinhead to a walnut and also some perirenal tissue cysts. The whole organ was about twice the normal size. The cysts were all punctured.

An x-ray report stated that "a second examination of the right side failed to show the outline of the right kidney. No tumor mass was seen. Colon plates show the hepatic flexure and transverse colon pushed downward by a tumor mass."

[In March, 1915, the patient had been in the hospital and at that time a functional test (phenolsulphonphthalein) showed a normal output of dye, but decreased fluid on the right. At that time operation was refused.]

About three weeks after the operation, while the patient was stooping over a pan washing her face, something seemed to break inside of her, and from this time she has had pain and has been worried. She said that during the past summer she was "puffed up all over her stomach," and could not eat. The week before the last admission she had pain on urination and some bleeding which made her feel better. She has had to urinate two or three times during the night since this time, whereas previously she used to get up every 15 minutes. Now she has chills, irregularly, several times a week (five to six times). With these chills, which are nocturnal, she has no renal pain or sweating.

PRESENT STATE.—The patient is a thin, elderly woman lying quietly in bed. Her eyes react to light and accommodation. The nose, eyes, ears and neck are negative. The tongue appears normal. The chest shows symmetrical expansion and resonance. Over the right lung there are no râles heard, and no evidence of consolidation, but there is a friction rub over the middle lobe. The cardiac rhythm is normal, and the pulses equal and even. There is abdominal tenderness over both kidneys. The right kidney seems to be enlarged and cystic.

Cystoscopic examination shows that the mucous membrane of the bladder is fuzzy

\*From the Mary M. Emery Department of Pathology of the University of Cincinnati, and the Pathologic Institute of the Cincinnati General Hospital.



in appearance, with alternating reddish and whitish areas. There is one dark hemorrhagic area above the ridge. The ureters are hard to catheterize; the right gives a rapid flow of clear urine; the left a small amount. Functional test with carmine red gave no results through the left kidney in 30 minutes.

*February 7.*—There is a large mass in the right renal region which is not related to the liver dullness, and which feels cystic. It is about twice the size of a normal kidney. A catheterized specimen of urine showed albumin for both sides, many leucocytes, a moderate number of red cells, and a few casts.

*February 12.*—The patient complains of frequent and burning urination.

*February 14.*—She is weaker and has pain in the left side.

*February 18.*—Died.

CLINICAL DIAGNOSIS.—Double cystic kidneys.

#### AUTOPSY PROTOCOL.

The body was that of a slender, emaciated, coffee-colored negress, with not very kinky hair. Rigor mortis was present; postmortem lividity was faint. There was a slight edema of the ankles. The peripheral lymph glands were not appreciably enlarged. The pupils were equal. The conjunctivæ were pale. The upper teeth were replaced by an artificial plate. All except the anterior lower teeth were missing and those that remained were in good condition. In the right flank posteriorly was an old atrophic incision 14 cm. long, that reached from the lower border of the last rib to the crest of the ilium 8 cm. posterior to the anterior superior spine. The breasts were atrophic and flaccid and contained no nodules. There were lineæ albicantes on the abdomen above the pubis and on the upper anterior surface of the thighs. The lower margin of the liver lay  $8\frac{1}{2}$  cm. below the ensiform and  $3\frac{1}{2}$  cm. beneath the costal margin in the right mammillary line. The omentum was coiled into a solid strand chiefly above the transverse colon and was punctated with innumerable fine hyaline and gray nodules that resembled miliary tubercles. It was adherent to the abdominal wall above the left inguinal ring. The peritoneum everywhere over the surface of the intestines and over the parietes was studded with fine translucent nodules resembling miliary tubercles, many of which were pigmented, the pigment forming, as a rule, a ring about the tubercles. The appendix was *in situ* and, except for the presence of the tubercles upon its surface, seemed to be healthy. In the pelvis the tubercles were larger, less discrete, and far more numerous than elsewhere in the abdominal cavity. There was no increased peritoneal fluid. When the sternum was removed, the lungs did not collapse, largely because both pleural cavities were completely obliterated with old adhesions. The right kidney was adherent by old adhesions to the hepatic flexure of the colon and the left kidney was adherent by old adhesions to the splenic flexure. There were old adhesions between the dome of the liver and the diaphragm and in these adhesions were very numerous tubercles. The mesenteric and retroperitoneal lymph glands were all hyperplastic, distinctly pale and moist, and in many of them there were areas that appeared to be due to caseation.

The right kidney (1,010 grams), was firm and seemed to be composed almost entirely of a large number of cysts varying in size from a few millimeters in diameter to several centimeters. The capsule was apparently completely adherent and on section it appeared that some of the cysts were filled with a rather clear, almost serous fluid; others contained an inspissated, dark colored material containing blood; and still others contained a material rather thick like pus. There were other areas which were not cystic which were filled with a yellowish caseous material and these areas were typically tuberculous. The left kidney had the same gross appearance but on section showed no evidence of tuberculous process but had all the appearances of a typical cystic kidney. The liver was of fair size but very flabby. The surface was scarred almost generally with the tags of old adhesions. Scattered here and there throughout the substance in both lobes were very numerous subcapsular cysts, all, so far as could be seen, filled with clear fluid. One of these cysts, the largest, measured  $5\frac{1}{2}$  cm. in diameter. There were occasional areas that resembled small caseous spaces. There were occasional areas that resembled small caseous tubercles. One of these seemed to have originated in the neighborhood of a bile vessel for it had a central greenish nucleus. The gall bladder was filled with a mucoid brownish bile and the ducts were patent.

The lungs, after removal from the body, were almost completely collapsed. The

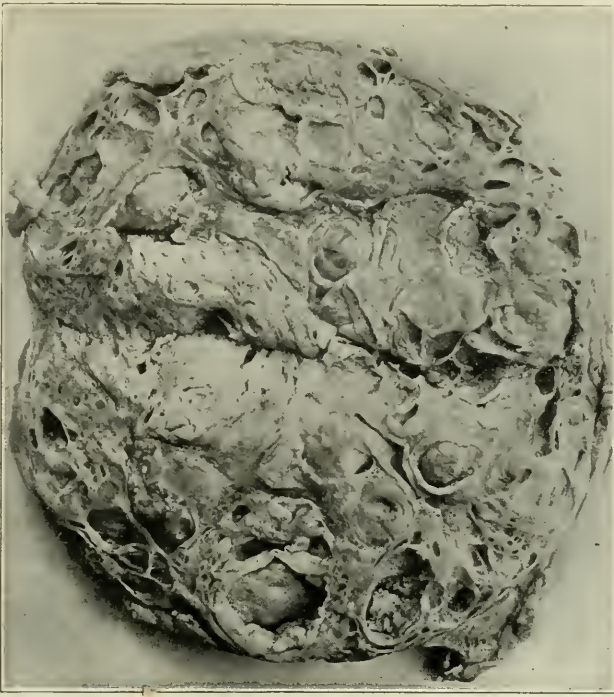


Fig. 1.—Photograph of the right kidney showing the cysts, some of them filled with caseous material or tubercles, others patent. Many small, almost miliary tubercles are also visible.

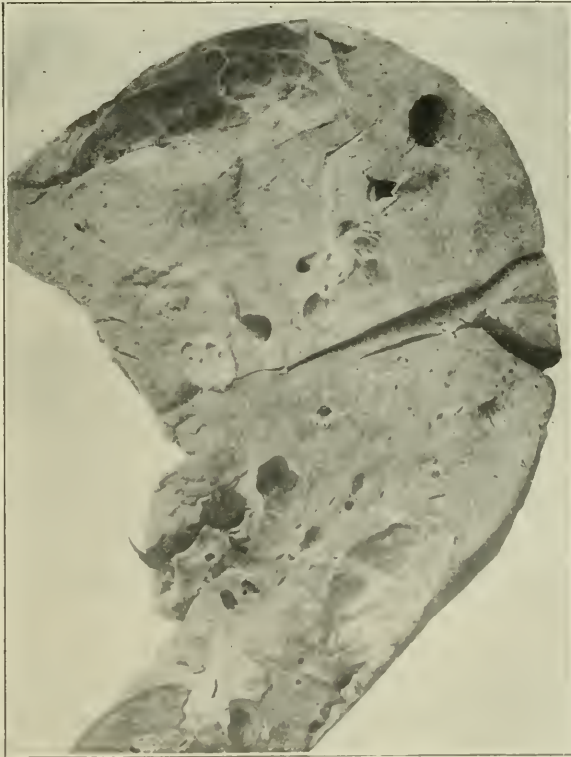


Fig. 2.—Photograph of a section of the liver to show the thin-walled, somewhat trabeculated cystic spaces.

pleura was generally thickened and beneath it and in the substance of both upper lobes, there were numerous firm, fibroid nodules which on section seemed to be obsolescent pigmented calcareous tubercles. There were a few such nodules in the lower lobes. The lower lobes were bound together by old adhesions. The heart was small and flabby. The pericardium was smooth. The coronaries were slightly tortuous but not evidently sclerotic. There was nothing unusual in connection with the tricuspid or pulmonary orifices. The foramen ovale was open but protected by a competent valve. The mitral and aortic valves were healthy. The aorta was smooth throughout and showed nothing more than scattered patches of subintimal fatty degeneration. The myocardium was pale and friable. There was no evidence of fibrosis. The spleen was of normal size. The surface was tagged with old adhesions and the color was pale. On section, the pulp was distinctly pale and the consistency was normal. The Malpighian bodies were not visible and at one or two points there were small gray areas which resembled tubercles. Both fallopian tubes were convoluted and the convolutions were bound together by old adhesions. Also both tubes were large, the enlargement being apparently due to increased semisolid contents. Except that the ovaries were more or less surrounded by old adhesions there was nothing abnormal about them. The uterus was of normal size.

The urinary bladder contained a small amount of cloudy blood-tinged purulent urine. The whole mucous membrane had an irregularly congested and generally moist appearance. The left ureter was evidently healthy. The right ureter, in its lower 7 cm., was the seat of a well marked, chronic, ulcerative tuberculous process. The right fallopian tube appeared to be a practically solid mass of caseous material. The left tube was partly caseous, partly suppurative. Around the cervical opening of the uterus there was a slight amount of erosion but nothing else. The vagina and body of the uterus seemed to be healthy except for the presence of a few small fibromas in the myometrium. The mucous membrane and submucosa of the rectum were edematous but showed nothing else unusual. In the large intestine there was nothing unusual except a moderate edema of the muscular coat. In the small intestine there were numerous ulcers. In the cecum there was one very large transverse, evidently tuberculous, ulcer which measured 7x3 cm., the base of which was congested and in places covered with a rather adherent, greenish colored pseudomembrane. Above the cecum in the first 10 cm. of the ileum, were very numerous small ulcers with thickened edges in which one could see occasional tubercles. Above this there were other scattered ulcers most of them small but all of them more acute than the cecal ulcer.

**ANATOMIC DIAGNOSIS.**—Congenital cystic kidneys and liver; chronic tuberculous nephritis; chronic tuberculous salpingitis; chronic and acute tuberculous enterocolitis; chronic tuberculous cystitis and (right) ureteritis; miliary tuberculous peritonitis; chronic miliary tuberculous lymphadenitis; obsolescent pulmonary tuberculosis; miliary tuberculous hepatitis and splenitis.

#### REMARKS.

It is interesting to speculate upon the course of the tuberculous process in this case, and to wonder whether it originated in the lungs or in the urogenital tract. The evidence furnished by the distribution of the peritoneal lesion tends to make it clear that the intestinal tract was not primarily involved for the peritoneal tubercles did not show the peculiarity of distribution which goes with intestinal ulcers; there was no grouping of the tubercles below the ulcers in the peritoneum. It seems most plausible to imagine that the process began with a pulmonary lesion which healed after bacilli had been distributed to the kidney and the fallopian tubes, in which places the process progressed.

The case illustrates exceedingly well how little kidney substance is required to preserve the health of the body. In these two enormous organs there was scarcely a macroscopic bit of kidney parenchyma, and yet up to the time the patient was overwhelmed by the tuberculous process, she lived in apparent comfort and health.



## LABORATORY METHODS

---

### THE EFFECT OF THE NATURAL ANTISHEEP HEMOLYSIN CONTENT OF HUMAN SERUM ON COMPLEMENT- FIXATION TESTS\*

BY ANNA I. VAN SAUN, ALBANY, N. Y.

---

ONE of the principal objections to the antisheep hemolytic system most commonly used by serologists for complement-fixation tests is the fact that nearly all specimens of human sera contain natural antisheep hemolysin. This natural antisheep hemolysin is sometimes present in the patient's serum in such large amounts that the addition of artificial antisheep hemolysin is unnecessary for the production of hemolysis in the serum control tubes and many workers have found that in some cases this natural hemolysin will obscure evidence of specific fixation in complement-fixation tests.†

A number of modifications of the original Wassermann test have been devised to overcome this difficulty as, for example, that of Noguchi,<sup>2</sup> which uses an antihuman system, that of Hecht-Weinberg<sup>3</sup> which makes use of both the complement and antisheep hemolysin present in the patient's serum, and that of Bauer<sup>4</sup> which also depends on the presence of natural antisheep hemolysin in the patient's serum.

Kaliski,<sup>5</sup> in 1910, instituted a modification of the Bauer test which he describes as follows: "The tubes containing the serum to be tested, complement and antigen extract are incubated, emulsion of sheep's corpuscles added and incubated again. To the tubes showing no hemolysis or only partial hemolysis, two units of artificial amboceptor are added and the tubes placed in the incubator again. After the second incubation the presence of antisheep amboceptor is indicated by the degree of hemolysis."

The absorption method of Rossi<sup>6</sup> has been very generally used as a means of getting rid of natural hemolysin in serum. Olmstead,<sup>7</sup> in 1914, found through a series of comparative tests on fifty-two sera treated by this method, and tested by the method used by McNeil<sup>8</sup> in the Research Laboratory, Department of Health, New York City, that the natural amboceptor content of human serum makes but little difference in the Wassermann test if the readings are made as soon as serum and antigen controls are completely hemolyzed.

In the course of two years' experience in the State Laboratory with over

---

\*From the Division of Laboratories and Research, New York State Department of Health, Albany, N. Y.

†In a recent article on the Wassermann test Ottenberg<sup>1</sup> states that this natural antisheep hemolysin content of human sera is far more often demonstrated when a fixed amount (so-called excess) of complement (one-tenth c.c.) is used for the test and the balance of the system determined by means of an amboceptor titration as Wassermann and his followers advocate. Ottenberg seems to find that complement is more often overactive than underactive, and advocates a standardization of the system by means of a complement titration. In my experience I have found just the opposite to be the case. I have very seldom observed a mixture of complement that would titrate higher than a 1 in 10 dilution. Usually the titer would run about 1 in 8 and my standardization of the system by means of an amboceptor titration has given excellent results.

thirty thousand complement-fixation tests on more than fifteen thousand sera sent me for diagnosis by the Wassermann and gonococcus complement-fixation tests I found that a large number of sera possessed some natural antisheep hemolysin. I, therefore, considered it advisable to make a series of tests by the method of Kaliski, and the absorption method of Rossi, for comparison with my own method which is a modification of the original Wassermann technic in that the total volume of the test is .5 c.c. A number of comparative Wassermann tests were also made with another laboratory which used the antihuman system of Noguchi.

All reagents were carefully standardized daily. The tests were set up in duplicate. Complement was fixed for four hours in the ice box with the crude alcoholic antigen, or for one-half hour in the water-bath at 37° C. with the cholesterinized antigen, and readings made according to the method advocated by Citron.<sup>9</sup> Sixty-seven sera were tested by the Kaliski method which gave no better results than my own method. In no case did sera treated by the absorption method of Rossi give complete fixation of complement in the Wassermann test where before tests on the same sera had been negative. In a few instances weak reactions became somewhat stronger, but in no case did they become positive.

In the gonococcus complement-fixation test I have found it advisable to use a very delicate system in order to obtain the best results and in these tests the natural hemolysin content of the serum did occasionally obscure complete fixation of complement. For example: a one plus obtained with a serum with a large natural hemolysin content sometimes gave a two plus or three plus after absorption and very seldom a four plus. No negative, however, became positive, though occasionally a negative became plus minus. Some sera became anti-complementary and in a few instances the antibody content of the serum seemed weakened by the absorption treatment in spite of the care taken to remove every particle of saline from the packed cells.

Remarkably constant results were obtained with the comparative tests made with the laboratory using the Noguchi method. Although readings were made on an entirely different scale in each laboratory there were inappreciable differences in the end results.

#### CONCLUSIONS.

In these studies, as in those of nearly all previous investigators, the presence of natural antisheep hemolysin was demonstrated in a large proportion (in this instance 51 per cent) of the sera tested. Contrary to the results obtained by many students of serologic problems, my experiments show that the effect of natural antisheep hemolysin in the practical operation of the Wassermann test is negligible. In no instance did this excess of natural antisheep hemolysin obscure complete fixation of complement. There seemed to be little difference in the results of Wassermann tests with sera from which the natural hemolysin had been removed and in the results of tests made by my usual method. The results with the Kaliski modification showed no improvement over those obtained with the usual test, and comparative tests made with the laboratory using an anti-human system showed no advantage over my own.

In the case of the gonococcus complement-fixation test, it would seem to be advisable to absorb the natural hemolysin from sera showing a very great ex-



cess, since stronger specific fixations have in some instances been obtained with sera treated by this method.

## BIBLIOGRAPHY.

- <sup>1</sup>Ottenberg, R.: Arch. Int. Med., 1917, xix, 475.
- <sup>2</sup>Noguchi, H.: Serum Diagnosis of Syphilis, 1912.
- <sup>3</sup>Hecht: Wien. klin. Wchnschr., 1909, xxii, 265.
- <sup>4</sup>Bauer, J.: Deutsch. med. Wchnschr., 1908, xxxiv, 698.
- <sup>5</sup>Kaliski, J.: Arch. Int. Med., 1910, vi, 215.
- <sup>6</sup>Rossi, O.: Ztschr. f. Immunitätsforsch. u. Exper. Therap., 1911, x, 321.
- <sup>7</sup>Olmstead, M. P.: Med. Record, New York, 1914, lxxx, 341.
- <sup>8</sup>McNeil, A.: Collected Studies, Research Laboratory, Department of Health, New York, 1912-1913, vii, 325.
- <sup>9</sup>Citron: Immunity, 1914.

## THE WASSERMANN REACTION WITH LARGE AMOUNTS OF PATIENT'S SERUM\*

BY ANNA I. VAN SAUN, ALBANY, N. Y.

SOME serologic workers have attempted to show that more specific results can be obtained with the use of large amounts of human sera for the Wassermann test than with the amounts in ordinary use.

In this connection B. Fischer,<sup>1</sup> of the University of Rostock, Germany, cites the work of Ledermann<sup>2</sup> and von Kromayer Trinchese<sup>3</sup> as the basis for a series of Wassermann tests with large quantities of sera made by him at the Universitäts-Hautklinik, in Rostock. For two years he tested upwards of 1300 sera, using the Kromayer modification; that is to say, quantities of sera ranging from .1 c.c. up to .4 c.c. and he came to the following conclusions:

A. Large amounts of serum are capable of

1. Changing a negative Wassermann reaction to a complete positive.
2. Changing a slight inhibition to a positive reaction.
3. Giving an indicator for the cessation of treatment. (The latter only to be discontinued when the largest amount of serum, .4 c.c. gives a negative reaction.)

B. All reactions giving complete inhibition of hemolysis with .4 c.c. of serum are specific.

Fischer made use of a serum control of .4 c.c. (the largest amount of serum used in his test) which, he asserts, was never anticomplementary. This seemed to me an unsafe amount upon which to rely as an absolute control for so large a quantity of serum, since it is well known that very large amounts of serum have in themselves a tendency to bind complement without the presence of a specific antigen.

For my work all complement-fixation tests are controlled, not only by the largest amount of serum used for diagnosis, but also by double the largest amount of serum used in the test.

I, therefore, thought it might be of interest to test a number of sera for the Wassermann reaction, not only with our standard amounts of sera, but also

\*From the Division of Laboratories and Research, New York State Department of Health, Albany, N. Y.

with the amounts recommended by Fischer with the addition of a double control of .8 c.c. of serum in order to determine the possible specificity of this reaction. Seventy-four sera out of eight hundred specimens received during a period of one month were tested according to the Kromayer modification as worked out by Fischer, using .4 c.c. of serum as our largest diagnostic amount, with an added control of .8 c.c. of serum, as well as by our own method, based on the original Wassermann technic, making use of two quantities of serum, .2 c.c. and .1 c.c., and controlled by .2 c.c. and .4 c.c. of serum. No test was considered safe to read until the double controls .4 c.c. and .8 c.c. respectively had hemolyzed completely.

In almost every case complete hemolysis did not occur with .8 c.c. of serum in the double control tube in the Kromayer test even though it was left for an hour in the water-bath. Very frequently the .4 c.c. control hemolyzed so slowly as to make me doubt the specificity of a reaction obtained with specimens from cases having no history of syphilis and where my own test with smaller quantities of serum was negative. No negative became positive, and slight fixations with the ordinary amounts of sera could in few cases be considered specific when stronger with the largest amount of serum since the double control of .8 c.c. in nearly every instance did not hemolyze completely.

Sera from cases with positive histories which gave complete fixation of complement with the larger quantity of patient's serum and which showed hemolysis in the .8 c.c. control were also complete with the smaller quantities.

Fischer gives a great deal of clinical data which appears to check his results, but I believe from my experience that the Kromayer modification would be decidedly unsafe to use in a laboratory where there is no close connection with the clinical side of the cases, and where histories are frequently vague, unless a double serum control is put in with every test and the diagnostic tubes read only when the double serum controls are completely hemolyzed; a result which, according to the findings on the number of tests we have been able to examine with the Kromayer modification, would be difficult to achieve.

#### CONCLUSIONS.

1. The use of a large amount of serum does not, in my experience, change a serum giving a negative reaction to one giving a positive if a double serum control (.8 c.c.) is used and the result of the test read only when this control is completely hemolyzed.

2. In a number of tests I have found that the .4 c.c. serum control has been extremely slow to hemolyze, and has occasionally fixed complement completely. This result appears to point to a lack of specificity in a test depending on this amount for diagnosis.

3. Since large amounts of serum may of themselves bind complement without the addition of a Wassermann antigen, and since, in the above tests, in almost every instance, controls of double these large amounts of serum did not hemolyze completely, I do not consider the Kromayer modification a safe method to use for the practical examination of large numbers of sera.

#### BIBLIOGRAPHY.

<sup>1</sup>Fischer, B.: *Deutsch. med. Wchnschr.*, 1916, xlii, No. 5, 135.

<sup>2</sup>Ledermann: *Berl. Dermat. Gesellsch.*, 1912, 10, xii.

<sup>3</sup>von Kromayer Trinchese: *Med. Klin.*, 1912, viii, 404.

# A METHOD FOR MAKING GRAPHIC RECORDS OF THE MOVEMENTS OF CERTAIN INTERNAL ORGANS

BY D. E. JACKSON, M.D., ST. LOUIS, MO.

THE instrument (Fig. 1) described in the following paragraphs has been used in dogs to record contractions of the stomach or pylorus (Fig. 2), the small or large intestine (Fig. 3), the uterus (Figs. 4 and 5), rectum, heart and bladder. In addition to these, the instrument can be used to register movements in the esophagus, and perhaps in the gall bladder, ureters, and spleen, although I have not as yet taken the trouble to thoroughly try the instrument out with the latter organs. These statements all refer to contractions recorded from the organs *in situ*, and in practically their normal positions. It is important that the blood supply and innervation to the organs from which records are being taken be not disturbed more than can be helped. In most cases this object is easily attained in using the instrument, for it has been designed with this end especially in view. In the abdomen the organs from which tracings are being

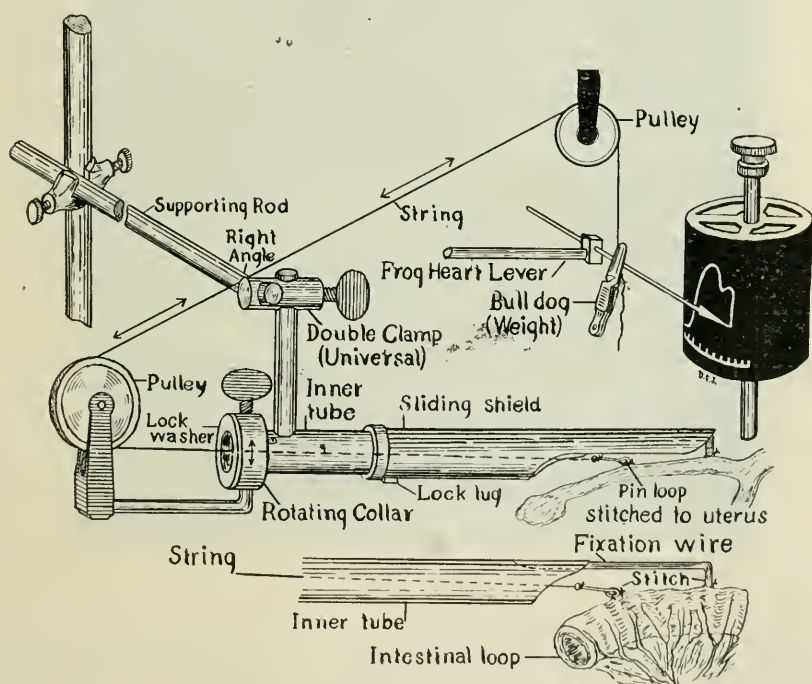


Fig. 1.—A diagrammatic representation of the method for using the instrument. (For discussion see text)

obtained may be left almost, if not entirely, covered with the other viscera; and thus marked changes in temperature, drying, etc., are almost entirely avoided. Usually the abdomen can be closed by hemostats or stitches and the vitality of the animal is thus greatly conserved. In addition, shock is much less likely to

\*From the Department of Pharmacology of Washington University Medical School, St. Louis, Mo.



occur than if the abdominal organs are considerably exposed to the air or otherwise extensively manipulated.

It is very easy to attach the instrument to the organs. In the abdomen this is done by first making a median longitudinal incision. In case the uterus is to be used one horn is then carefully exposed by moving the intestines up toward the diaphragm. Great care should be used not to touch the vessels or nerves going to the uterine horn. One may readily avoid these since they run along the posterior (caudal) edges of the organ. When one horn (usually the left) is brought into view, the flank of the animal is held up by a hemostat and

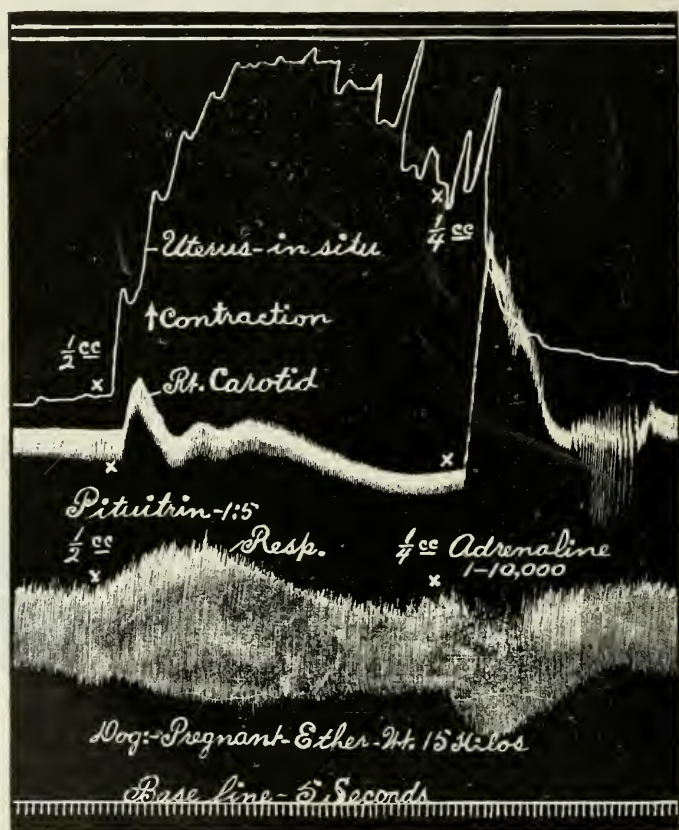


Fig. 2.—A tracing showing the manner in which the instrument records uterine contractions after pituitrin (and adrenaline).

an opening about one and one-half inches long is cut through the lateral abdominal wall about an inch below (caudalward from) the left ribs. The instrument is then clamped in a stand in such a manner that the two tube portions ("inner tube" and "sliding shield") should have a direction pointing caudalward, with reference to the animal, at an angle of about forty-five degrees from the median line, and inclined upward at a considerably smaller angle. The inner tube portion should then be about on a level with the exposed uterine horn. The tube portion of the instrument is then passed through the opening in the animal's flank by shoving the stand which holds the instrument closer to the animal. In



this manner the instrument is brought into a position parallel to and in immediate relation with the uterine horn. Before the instrument is inserted into the abdomen a long twine string carrying a pin with a ring bent on the end is passed over the pulley and through the length of the tube. When the tube is pushed into the abdomen the ring on the pin hook is then seized with forceps,

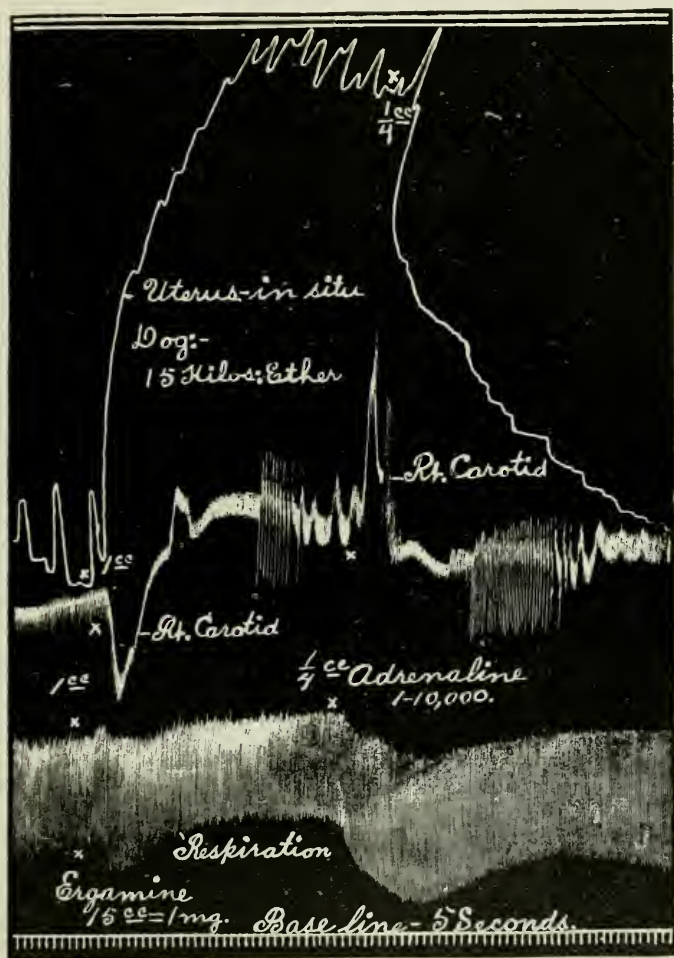


Fig. 3.—A tracing showing a record obtained with the instrument after ergamine (and adrenaline).

and with a single stitch made with great care with a fine, curved-pointed surgeon's needle the ring is tied to the upper edge of the uterine horn at a distance of about one and one-fourth inches from the bifurcation. During this process the "sliding shield" (Fig. 1) is slipped back on the inner tube so as to fully expose the "fixation wire." As soon as the ring or the pin has been attached, the bent end of the "fixation wire" is brought into position and attached with a single stitch to the tissues at the upper edge of the bifurcation. Thus the nerves and vessels to the horn need not be touched and the instrument is quickly and easily attached. The "sliding shield" is now slipped down over the "fixation wire" and the string is put on a little tension to see that a small mass of tissue

does not block its movement at the end of the inner tube. The abdomen is now completely closed, either with hemostats or stitches. The "sliding shield" serves to keep the other viscera from moving the uterine horn while records are being taken. The string is now passed over a pulley and connected to a frog heart lever which writes on the drum. The magnification of the lever should be about one to eight, or one to ten. The weight suitable for good sized bitches is about two or three ordinary bull dog clamps. It is important not to include too long a portion of the uterine horn between the two attachments. About one and one-

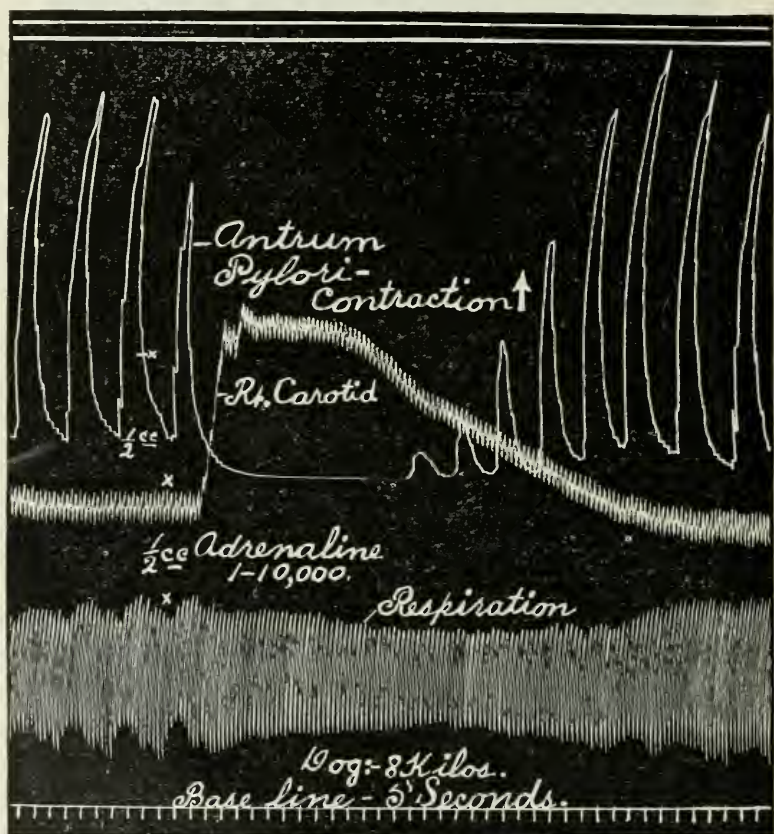


Fig. 4.—A tracing showing how the instrument may be used to record contractions of the antrum. The inhibitory action of adrenaline on the spontaneous contractions of the antrum is well shown.

fourth inches (or a little less) is approximately the length usually needed. When the record is started the chances are considerable that the uterus will already be in a condition of tonic contraction. To overcome this it is advisable to inject a small dose ( $\frac{1}{4}$  c.c. of 1:10,000) adrenaline to relax the organ. The drug produces a small primary contraction followed by marked relaxation (Figs. 2 and 3). A word of caution is necessary in regard to female dogs. Pups or young animals which have never borne young are not suitable for the experiment. Animals which are, or have been, pregnant previously are entirely satisfactory.

Records may easily be taken from almost any portion of the alimentary canal. For most parts of the stomach and intestines the instrument may be

readily brought into a satisfactory position through the median abdominal incision. Rarely one may wish to make a second small opening in the side of the abdominal wall for the insertion of the instrument. In practically all cases the abdomen should be securely closed after the instrument has been properly adjusted. It is of importance to note that records of either the longitudinal or the circular movements of the stomach and intestines can be obtained equally easily. This perhaps may be of use in studying the action of drugs, etc., on the various forms of peristalsis.

Fig. 4 shows a record obtained when the instrument was attached across, (i. e., transversely to) the antrum pylori. Spontaneous contractions of the antrum were occurring, but these were immediately inhibited for a brief interval by an injection of adrenaline. It is entirely feasible to attach two or three instru-

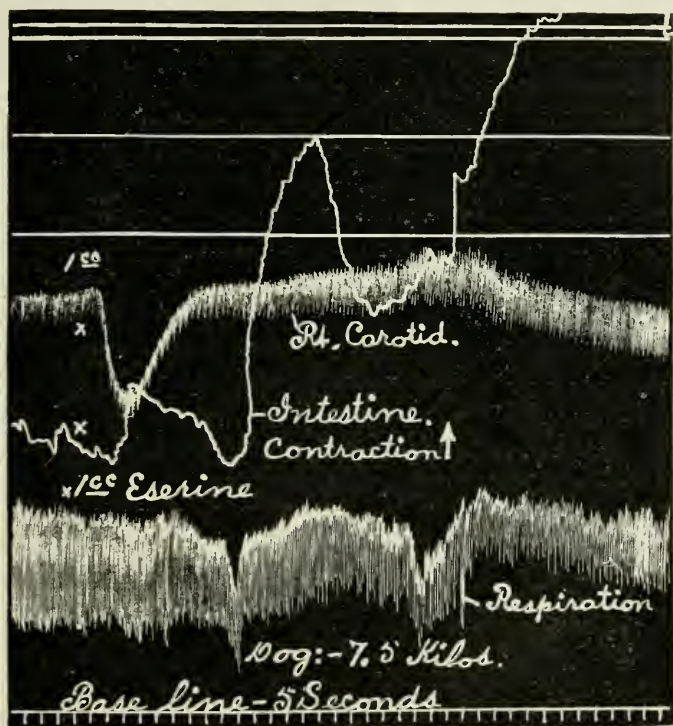


Fig. 5.—A tracing showing the action of eserine on the large intestine as recorded by the instrument.

ments to different portions of the stomach and thus obtain graphic records of the rate and character of its movements after vagus stimulation, etc.

Fig. 5 shows a tracing taken from the large intestine after an injection of eserine. It is to be noted that the large intestine, and particularly the lower portions, gives much larger and more constant contractions than does the small intestine. The record shown represents movements recorded from longitudinal attachments of the instrument. About one inch is usually a suitable length of intestine to include between the two stitches. The instrument may also be attached transversely to the intestine and two instruments may be used simultaneously if desired.



From the bladder records may be obtained by attaching the instrument either to the anterior, posterior, or lateral walls, or across the fundus of the organ, and these attachments may be made either longitudinally or transversely. To record the action of the heart the chest should be opened directly in the median line and throughout the entire length of the sternum. Four heavy strings are passed through the edges of the sternum, two on each side, in such a manner that the chest can be held open about two inches when the strings are tied down to the side of the operating board. The pericardium is now opened and the divided edges are attached by one or two stitches on each side to the edge of the chest walls.

The instrument is now placed over the heart in an almost horizontal position and pointing caudalward, i. e., "the fixation wire" is sewed to the apex of the ventricle. The ring on the end of the pin is attached to the base of the ventricle. The shield is now slipped down and the chest may be partially or completely closed, while the string passes to the recording lever in the usual manner. It is very desirable to have a perfectly regular artificial respiration, and it is advisable to give the animal a hypnotic (paraldehyde or chloretone) to be sure it will not revive and disturb the adjustments of the apparatus by slight movement during the experiment.

While I have so far had an opportunity to try this instrument only on dogs, I anticipate that it can be used equally well for cats or rabbits in the experiments for which those animals are suitable.

The wide applicability of this instrument, together with its comparative inexpensiveness (the device, exclusive of the heart lever and extra pulley, should cost approximately ten dollars; the extra pulley can be bought in large hardware stores for about fifty cents) has seemed to indicate that it might be of considerable use to teachers of pharmacology and laboratory workers in general; hence the publication of this brief note.

---

## A SIMPLE METHOD OF OBTAINING BLOOD SERUM\*

BY M. G. WOHL, M.D., OMAHA, NEBR.

---

A SATISFACTORY separation of serum from blood is desirable if not essential for various serologic reactions. Not all bloods, however, yield a good contracted clot allowing the serum to separate. It becomes necessary then to loosen the blood clot from the walls of the container by means of a platinum or glass rod. This procedure is undesirable as the introduction of the rod is liable to break up the red blood corpuscles and discolor the serum by the liberated hemoglobin; and again, it causes delay in obtaining the serum.

To meet these objections, we have been using in our laboratory paraffined containers. Blood which is quite adherent to the walls of an ordinary container will yield a clear serum when it is placed into a paraffined vessel. That

\*From the Pathological Department, Nicholas Senn Hospital, Omaha, Nebr.



the paraffine does not alter the serum in any way, so far as the Wassermann and Widal reactions are concerned, we have proved by using controls of serums obtained in the conventional way. This method has been constantly used in our laboratory for the last three months and the results have been most gratifying.

#### TECHNIC.

Place a small piece of paraffine (half a gram is sufficient for an ordinary six inch serological test tube) into a container that is ready for sterilization, cork it and sterilize it. Upon removal of container from sterilizer roll it so as to cover its walls with the paraffine. It takes only a few minutes until the thin film of paraffine is hardened. To clean the container, rinse it with cold water until all the blood is removed, melt the paraffine and pour off. The excess of paraffine is removed by means of a gauze applicator. To the best of my knowledge paraffined containers have not been used heretofore for the above described purpose.

# *The Journal of Laboratory and Clinical Medicine*

VOL. III.

OCTOBER, 1917

No. 1

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.

Ann Arbor, Mich.

## ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D.	- - -	ST. LOUIS
HANS ZINSSER, M.D.	- - -	NEW YORK
PAUL G. WOOLLEY, M.D.	- - -	CINCINNATI
FREDERICK P. GAY, M.D.	- - -	BERKELEY, CAL.
J. J. R. MACLEOD, M.B.	- - -	CLEVELAND
ROY G. PEARCE, M.D.	- - -	CLEVELAND
ROGER S. MORRIS, M.D.	- - -	CINCINNATI
GERALD B. WEBB, M.D.	- - -	COLORADO SPRINGS
E. E. SOUTHARD, M.D.	- - -	BOSTON

Contents of this Journal Copyright, 1917, by The C. V. Mosby Company—All Rights Reserved  
Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

## EDITORIALS

### *The Use of Poisonous Gases in the Present War*

NOTWITHSTANDING the fact that the use of poisonous gases in war is distinctly prohibited by the Hague Convention, the Germans began the employment of this barbarous weapon early in 1915. At first it fell upon the unsuspecting Allied Armies like a blast from the furnace of death, but within a short time means of protection were found and since that time, the Allies have not hesitated to fight the devil with fire. To be efficient in war a gas must be not only toxic, but it must possess certain physical properties. It must be heavier than air, sufficiently dense to prevent rapid dissipation and it must retain its gaseous state within certain variations in pressure and temperature.

Poisonous gases so far employed in war may be divided into the following classes:

1. Those which are directly and immediately poisonous.
2. Those which produce slow death, by asphyxiation.
3. Those which put the victim out of action without doing permanent harm.

Those of the first class are hydrocyanic acid (HCN), arsenide of hydrogen ( $\text{AsH}_3$ ), sulphide of hydrogen ( $\text{H}_2\text{S}$ ), and phosphide of hydrogen ( $\text{PH}_3$ ).

Those of the second class are chlorine (Cl), oxychloride of carbon

(C-O-Cl<sub>2</sub>) known as phosgene, acroleine (CH<sub>2</sub>-CO-CHO) bromacetone (CH<sub>3</sub>-CO-CHBr<sub>2</sub>), chloropicrine (CCl<sub>3</sub>-NO<sub>2</sub>), methyl chloride of chloroform (Cl-CO<sub>2</sub>CH<sub>2</sub>-Cl) and methyl chlorosulphate (SO<sub>2</sub>-OCH<sub>3</sub>-Cl).

Some of the third class are chloracetone, bromacetone, iodacetone, bromacetic ether and iodacetic ether. Those of the third class are not known as poisonous gases, but as lachrymogenous. They cause an abundant flow of tears and for the time disable the men.

The Germans first used chlorine and the French replied by the use of phosgene (the oxychloride of carbon) which is said to be twenty-four times as effective as chlorine.

Poisonous gases are used, (1) in waves, (2) in hand grenades, (3) in shells. To these may be added the employment of liquid fire. Hand grenades filled with hydrogen phosphide and high explosives have been used. When exploded they emit a dense cloud of burning phosphorus. Chlorine and its compounds are usually employed in waves. On account of its lightness, phosgene is mixed with chlorine, and this mixture is known as "Collongite." When released from the tanks, the gas rolls along the ground and settles in the trenches and dug-outs. The effectiveness of the wave depends greatly upon weather conditions, notably upon, (1) wind, (2) humidity, (3) sunshine. The wind must be in the right direction and must not be too strong. A wind of about six meters per second or thirteen miles per hour is most suitable. A humidity of from forty to sixty per cent is most favorable. In bright sunshine and with low humidity, the gas is soon dispersed. Along the western front daily weather observations are made and forecasts recorded. The working of a gas attack is under a specially trained brigade of engineers. The tanks are so placed that the gas will reach the enemy and not fall into their own trenches. Since the trenches are tortuous and communicating, this is often no small task. The tanks are about ten inches in diameter and four feet long, weight when filled about 160 pounds and are fitted with folding handles. One great desideratum is to surprise the enemy and many devices have been resorted to for this purpose. Concentration of the gas is a matter of the greatest importance. When the Germans first used the gas it killed some who were ten kilometers to the rear. The liberation of a gas wave is accompanied by artillery firing and followed by an infantry attack. This must be wisely correlated or disaster will come to the attacking side. One purpose of the artillery fire is to cover the noise of the gas escaping from the tanks. Several plans have been devised for the early detection of the approaching gas. Strips of paper which are turned red by traces of chlorine are suspended in front of the trenches. A flame on the principle of a Bunsen burner plays upon a ring of metallic copper. The smallest trace of chlorine produces a green color.

Much effort has been expended upon devices to secure protection against the waves. High explosives have been tried in attempting to disperse the wave but without satisfactory results. Neutralizing gases and liquids have also proved ineffectual. Fire pots along the edge of the trenches were used for a while, but have been for the most part discontinued. These pots are ignited when the gas alarm is sounded and do something in the way of dispersing and lifting the gas. Bags filled with sawdust and ignited with oil have been used

for the same purpose. Sprays of hyposulphite, bicarbonate and carbonate of soda have been used. All attempts to secure collective protection against the chlorine gases have failed and for the most part have been discarded and reliance is now placed upon the individual mask.

The latest French mask is made of several thicknesses of gauze quilted together, consisting of a front and a crescent-shaped piece which, when the mask is adjusted, fits from just in front of the ears, down the cheeks and under the chin, the two horns of the crescent being in front of the ears. The front carries two goggles of celluloid fixed in place by metallic rings and inside rubber washers. The mask is held in position by elastic bands and tape. The band which goes around the back of the head holds the front firmly against the forehead, while that passing laterally over the head, attached to the crescents, pulls tightly up under the chin.

The gauze of which this mask is made is light brown on the outside and light green on the inner side. The gauze is impregnated with the neutralizing solution and furnishes complete protection for four hours when the gas is present in the proportion of 1:1000. It affords no protection against carbon monoxide. When not in use the mask is placed in a waterproof case and suspended like a haversack. The mask weighs 375 gm. and the cost is about sixty cents.

The Tissot apparatus, furnished the gunners, is more elaborate than the mask and permits better vision. It consists of a metal box about 14 x 6 inches, which carries the chemicals and is suspended between the shoulders. A flexible tube ending in a mask passes over the shoulder. The inspired air passes through the chemicals in the box and is purified. Valves are so arranged that the expired air is discharged outside the mask. The air first passes over water by which it is moistened, then sodium carbonate which neutralizes the chlorine, then sodium peroxide which supplies oxygen. The English have a similar apparatus which is carried over the left shoulder and is swung around to the chest when in use.

The only protection against carbon monoxide so far used is the Draegen apparatus, long used in mines. It consists of a cylinder of oxygen connected with a rubber bag with a tube terminating in a mouth piece. The apparatus is suspended from the neck and hangs in front. The nose is closed by a clip. Horses are protected by masks, and guns from the corroding effects of chlorine gas by vaseline or some heavy oil. The charging of shells with liquefied gas is a delicate operation, and must be done at low temperature. The No. 4 French gas shell contains the following mixture:

HCN	50 parts
Sn Cl <sub>4</sub>	35 "
As Cl <sub>3</sub>	10 "
CHCl <sub>3</sub>	5 "

The tin chloride seems to keep the hydrocyanic acid gas at proper tension. The arsenic is added as a smoke producer and the chloroform prevents crystallization. No. 5 shell contains phosgene and No. 12, bromide of benzyl. The last mentioned is especially valuable in "Counter Battery" work in which No. 12 shells are alternated with high explosives.



The liquid fire apparatus consists of a compressed air tank under a pressure of 600 kilos per square meter connected by an iron pipe with a valve with a second tank containing gasoline under a pressure of 10 kilos per square meter. From the gasoline tank a reinforced flexible tube passes under the right arm and ends in an iron projection with a pilot flame. The operator opens the valve between the two tanks in his back, lights the pilot by a friction slide and ejects the flame in any direction and up to 20 meters. With a continuous flame, one tank lasts about ten minutes and with an intermittent flame, much longer. The gasoline may be used as a spray and then ignited.

Professor Archard has prepared an official pamphlet on the use of poisonous gases from the medical standpoint. From this the following abstract is made.

Medical officers should familiarize themselves practically with wearing the masks in rooms filled with gas and in observations on animals both partially and fatally gased. Both French and English officers have this practical training. This is done both in rooms and in the open with conditions as nearly identical as possible with those of actual warfare. The first effect of small quantities of chlorine (1:1000) is irritation of the upper respiratory tract causing coughing and a sensation of suffocation. Rarely this may cause unconsciousness resembling chloroform anesthesia. Autopsies have been made on men found dead in the trenches, as the result of a wave, and showing no pulmonary lesion. This is an exceptional condition. In stronger concentrations of chlorine, men surprised, feel an intense suffocation, fall helpless and die in a few minutes. The face is dark, the lips greenish blue, the skin greenish with spots that are almost black. Experimentally dogs fall within 45 seconds and are dead within twice this time. On immediate autopsy the peripheral vessels do not bleed. The lungs do not occupy more than one-third the chest cavity. In color they are greenish gray and in consistency that of India rubber. On section the lungs are dry, bloodless and friable, having the appearance of cooked sausage. The trachea and bronchia are dry and gray. The heart is in systole, dry and rough. The right heart is slightly dilated and contains blood, which is thick and stains the hands brown. Other organs appear normal. Rapid death is frequent among the unprotected. In slower poisoning there is quickly developed an edema of the lungs and death may occur within an hour or after some days. One hears over the entire lung area, the subcrepitant rales of edema though this may be marked more or less by stronger bronchial rales. The heart sounds are heard with difficulty on account of the respiratory sounds. The median pulse may be imperceptible and the arterial tension falls way low. The individual often complains of severe pain in the base of the chest. Vomiting and purging may occur, but are not constant. Some die without the appearance of lesions and from severe intoxication. As a rule these are the older men.

Whatever may have been the beginning, on arrival at the hospital there is an incessant cough. Admission to a warm room and the recumbent position act as sedatives. Cold, speech, and movement intensify the cough. The temperature may be elevated one or two degrees for a few days but then falls to normal or below. Headache and asthenia are marked and may last for some weeks. As a rule the urine is normal but there may be suffusion followed by

albuminuria. A prognosis can not be safely made within three days but most of those who live this long recover under proper care. Old men are more seriously affected than the young.

One of the most common complications is a subcutaneous emphysema manifesting itself by a distension of the tissues, particularly at the base of the neck. Pulmonary gangrene is rare but gassed men should be kept out of hospitals in which surgical gangrene has developed. Gangrene of the extremities sometimes terminates in death. Poisoning with phosgene is the same as that with chlorine but more serious.

The blood is black and tarry and does not run well. There is no deformity of the cells, white or red. The latter are increased in number and show no abnormality in hemoglobin content or spectroscopic appearance.

The main points in the treatment of those poisoned with chlorine or phosgene are the following:

1. Stop the poisoning by removal from the vitiated air or by protection with a mask.

2. Assist normal aeration by giving a pearl of ether, by the inhalation of oxygen or by inhaling very dilute ammonia. The last mentioned procedure must be resorted to with the greatest caution.

3. Restore normal circulation and blood pressure by taking from 300 to 500 c.c. of blood and repeating this with due caution if necessary by subcutaneous injections of caffeine or strychnine. Subcutaneous injections of large volumes of fluid, salt solution, alkaline or hyposulphite solutions should never be resorted to.

4. Relieve the obstruction of the bronchi by emetic doses of ipecac. Smaller doses of this drug may be continued for some days.

5. Allay the irritation by rest in a warm, moist air with the inhalation of oxygen. It should be understood that any use of oxygen is only sedative and not curative. One may be poisoned with chlorine in the midst of an excess of oxygen.

Hydrocyanic acid gas kills but does not wound. There is an odor of bitter almonds. A sense of constriction about the throat and head and then unconsciousness. The body stiffens with a few tremors and death results. Pathologic changes are not marked. The tissues are reddened somewhat by the combination of the poison with the hemoglobin. The masks give some protection.

First aid stations should have all openings away from the enemy and in a gas attack double cloths should be hung over the entrance and kept sprayed with a solution of carbonate and hyposulphite soda—the same as is used in the mask. The clothes of all who enter the station in a gas attack should be treated with the same spray. The usual formula for the neutralizing solution is as follows:

Water	1000 parts
Hyposulphite of Soda	220 "
Carbonate of Soda (Solvay)	175 "
or Crystalline	475 "

—V. C. V.

# The Journal of Laboratory and Clinical Medicine

Vol. III.

ST. LOUIS, NOVEMBER, 1917

No. 2.

## ORIGINAL ARTICLES

### AN INVESTIGATION OF THE CHEMICAL COMPOSITION AND BIOLOGIC AVAILABILITY OF PEPTONE\*

By LEWIS DAVIS, S.M., DETROIT, MICH.

THE use of peptone in the preparation of bacteriologic culture media is advocated in the earliest works on bacteriology. While it is undoubtedly a fact that for many organisms ordinary beef infusion furnishes all of the necessary food requirements, addition of even a small amount of peptone causes a pronounced increase in growth and metabolic activity.

Bainbridge<sup>1</sup> and others have observed that even some of the extremely active putrefactive organisms are unable to attack and decompose native proteins. However, if a small quantity of peptone or some other readily assimilable, nitrogenous material be present, decomposition soon takes place with rapid disappearance of the protein.

That there is a specificity to the role of peptone in a culture medium, is particularly shown by its use in the elaboration of toxin by Bact. diphtheriae, the production of indol by the colon bacillus and its necessary presence for the successful growth *in vitro* of such delicate organisms as the spirochetes. The early investigations of Park and Williams<sup>2</sup> have pointed out the necessity of peptone in the production of diphtheria toxin. Recent successful use of media containing tryptophane<sup>3</sup> for the production of indol by bacteria clearly shows that the function of the peptone in Dunham's medium is to furnish material containing tryptophane.

It is readily apparent that the chemical composition of peptone has a decided influence on its bacteriologic availability. That this question has not received more attention in the past is largely due to the fact, that almost from the beginning of bacteriology, Witte's peptone has served as standard in culture media prepara-

\*From the Research Laboratory, Parke, Davis & Company, Detroit, Mich.

Read before the Laboratory Section, American Public Health Association, Cincinnati, Ohio, Oct. 24, 1916.

tion. The present scarcity of this imported peptone and the appearance of a number of domestic substitutes has necessitated careful consideration of their biologic availabilities. The present investigation was undertaken to compare the chemical composition as well as the utilization by bacteria of domestic peptones with Witte's peptone as a standard. In the course of the study, data was obtained which finally led to the preparation of an experimental peptone that has been found entirely satisfactory for bacteriologic use and in the production of toxins.

## EXPERIMENTATION

### A. *Methods of Analysis*

Samples of the various brands purchasable in the open market were obtained, each given a laboratory number for identification, and the examination was then conducted under the following captions:

1. *Physical examination* including appearance, odor, solubility in cold and hot water and preparation of the more common culture media from each sample, in accordance with the Standard Methods of the A. P. H. A.<sup>4</sup>

2. *Chemical examination* comprised analyses of total nitrogen, moisture, total mineral matter, phosphoric acid as  $P_2O_5$ , chlorides as NaCl, calcium as CaO and determination of reaction in aqueous solution. Supplementing the above, qualitative determinations were made for the presence of albumoses (addition of saturated zinc sulphate, ammonium sulphate, picric acid solutions) protoproteoses (addition of saturated sodium chloride solution, potassium ferrocyanide in acetic acid solution), tyrosin (xanthoproteic, Millon's reaction); tryptophane [(Adamkiewicz)-Hopkins-Cole reagent]; creatinin (Jaffe's reaction), the behavior towards the biuret test, as well as with three volumes of 95 per cent alcohol and a comparison of free amino acids present.

3. *Bacteriologic examination*, made in association with my colleague, H. C. Ward, included the determination of the conduct of various bacteria in the more common liquid and solid media made from each sample, growth and production of indol in Dunham's solution, viability tests with sensitive organisms, and direct isolation of bacteria from pathologic material. Of greater delicacy than any of the preceding tests, the writer has found to be the production of a potent toxin by *B. diphtheriæ* in ordinary (2 per cent) peptone bouillon. In fact, this test combined with development of indol by *B. coli* in Dunham's solution has been employed as "a preliminary test" of bacteriologic availability in the examination of experimental peptones.

For the analysis of total nitrogen, a modified Gunning method was used, which, while giving somewhat lower results than the official methods, was simpler and permitted of comparative results with equal accuracy. Moisture and total mineral matter were determined in platinum utensils in accordance with the usual technic. Phosphoric acid was estimated in a nitric acid solution of the ash by precipitation as ammonium phosphomolybdate, and dissolving in an excess of sodium hydroxide as employed in the volumetric estimation of total phosphoric acid in fertilizers.<sup>5</sup> The same solution of the ash was used for volumetric determinations of chlorides and calcium. The calcium was precipitated as oxalate, dissolved in sulphuric acid and titrated against potassium permanganate solution,



while the solution for chlorides was carefully neutralized with sodium hydroxide, and then titrated against silver nitrate solution with potassium chromate as indicator by Mohr's method.<sup>6</sup>

Estimating proteoses by precipitation with saturated zinc sulphate solution according to Bömer<sup>7</sup> was tried and gave fairly concordant results with Witte's peptone which, as is known, consists largely of proteoses. Most of the other samples examined had so little albumoses present that qualitative examination with the reagents mentioned above gave all the necessary information. Direct estimation of peptone nitrogen by phosphotungstic acid or bromine precipitation was found to be unsatisfactory. As the "tannin-salt method of Sjörning, as modified by Bigelow and Cook,<sup>8</sup> is dependent upon the proteose nitrogen value, this procedure could not be accurately applied for peptone valuation. Here again, the qualitative examination, supplemented by the biuret test, gave some idea of the degree of digestion of the sample.

The reaction of the sample peptones was determined on a 1 per cent aqueous solution both by a hot titration method and more accurately with the hydrogen electrode. For the hot titration, 10 c.c. of the 1 per cent peptone solution were diluted with 40 c.c. of water, boiled for one minute, then titrated hot against N/10 sodium hydroxide, using phenolphthalein as an indicator. The concentration of hydrogen-ions was measured by the hydrogen electrode recommended by Bovie,<sup>9</sup> using a Weston Standard Cell, calomel electrode, galvanometer, and the direct reading potentiometer described by Bartell.\* Comparison of the free alpha amino acids present in Witte's and the other test peptones was made with 1 per cent solutions, using the colorimetric method of Harding and MacLean<sup>10</sup> (color produced by twenty minute boiling with pyridine and ninhydrin) and a Duboscq colorimeter.

As test organisms for determining the nutrient index of peptone samples by subculture transfers, *B. coli*, *staphylococcus aureus*, *diplococcus meningitis*, *streptococcus pyogenes*, *M. catarrhalis*, *M. gonorrhoeae* and *B. prodigiosus* were employed. The growths on slant agar at 37° C. both after 24 hours and 48 hours were recorded for all except *B. prodigiosus* which was cultivated at 20° C. Inoculations from these were made to a new series of agar slants and the growths noted, after which the above process was twice repeated.

Dunham's solutions containing the test peptones (with 0.5 per cent sodium chloride) and sterilized at autoclave temperature, were used for growth and viability tests. Vigorous 24-hour, slant agar cultures of *Staph. aureus*, *B. coli*, and *B. prodigiosus* were emulsified with 10 c.c. of salt solution, filtered, and a uniform amount of suspension inoculated into a series of test peptone tubes by mixing with capillary and bubbling. A 1/10 c.c. sample was then removed from each tube and agar plates made as "controls." These were incubated at the optimum temperatures and counted. The peptone tube cultures were now grown for 24 hours and plates again made as before, the same procedure being repeated at 72 hours and 96 hours.

For the indol valuations, the same (Dunham's peptone) solutions as above

\*Paper read before the Biological Section, American Chemical Society, New York City, Sept. 27, 1916. This paper has now appeared in *Jour. Am. Chem. Soc.*, 1917, xxxix, 4, 630.

The work on hydrogen-ion concentration was done at the University of Michigan, Ann Arbor, in the laboratory of Prof. Bartell, to whom the writer's sincere thanks are due.

were employed. Four cultures of *B. coli* of known indol-producing capacity (with Witte's peptone) were selected, together with a culture of *B. typhosus* as a "negative control." Uniform, comparable, suspensions in sterile salt solution of a 24-hour slant agar culture of each of the organisms were made and 1 c.c. of each culture suspension added to sets of sample peptone tubes. The tubes were incubated at 37° C. for 96 hours, then qualitative indol determinations were made by adding 1 c.c. of a 10 per cent sulphuric acid solution, thoroughly mixing, and then adding without mixing 1 c.c. of a freshly made 0.01 per cent solution of sodium nitrite. The formation of the well-known purplish red, nitroso-indol ring of Salkowski<sup>11</sup> on standing in the cold for fifteen minutes indicated presence of indol, a record being made of the intensity of the ring. Although this test is not as delicate as the modified test of Ehrlich,<sup>12</sup> it was deemed of more practical significance, since for the case in hand indol should be formed in appreciable quantity to be of value as a diagnostic index.

Comment has already been made upon the importance of diphtheria toxin production in valuing a peptone sample. The test culture employed was of known toxin-producing power and gave rapid and abundant pellicle formation in ordinary 2 per cent peptone (Witte) bouillon. A reaction of the bouillon corresponding to a hydrogen-ion concentration of  $1 \times 10^{-5}$ \* (+5 on the Fuller Scale) before sterilization was found to give the best results. On sterilizing such a medium in an autoclave at 115° C. for 20 minutes, the reaction rises to about +10 on the Fuller Scale ( $C.H = 3 \times 10^{-8}$ ) which permits of rapid and vigorous growth.

In order to accustom the organism to any variations due to the test peptone, "starter" flasks containing 30 c.c. of bouillon in a 250 c.c. flask were first inoculated, cultured for 24 hours at 37° C., the purity of the growths checked, and the contents aseptically transferred to 3 liters of the same bouillon in 6 liter flasks. After incubating for 12 days at 37° C., examination for purity was made, 0.4 per cent trikresol added, allowed to stand 24 hours, then filtered.

Since diphtheria toxin is usually employed for immunization purposes, the strength of the toxins prepared as above was estimated by the L + dose in accordance with the Hygienic Laboratory Method.<sup>13</sup> An interesting fact noted in cultivating *Bact. diphtheriae* for toxin production was, that while the growth in larger flasks showed no gross difference from that obtained in small flasks, other conditions being the same, there was a marked variation in both toxicity and final reaction. Thus, 500 c.c. of bouillon in a liter flask invariably gave a weaker toxin and final alkaline reaction to phenolphthalein, while 3000 c.c. in a six liter flask gave a stronger toxin with final acid reaction to phenolphthalein. Large flasks were accordingly employed for all toxin cultivation with test peptones.

No preliminary cultivation was found necessary for the production of tetanus toxin. A virulent culture of *B. tetani* was used in glucose bouillon (2 per cent peptone) with oil, incubation carried on for three weeks at 37° C., examination for purity then made, and the same procedure followed as with diphtheria toxin. In accordance with the usual practice, the strength was determined by an approximate estimation of the minimum lethal dose, as recommended by Rosenau and Anderson.<sup>14</sup>

\*The relationship of hydrogen-ion concentration to toxigenicity of *Bact. diphtheriae* is being studied, and will appear in a later paper.

TABLE I  
PROPERTIES OF PEPTONE SAMPLES

AMERICAN BRANDS									
	No. 51	No. 67	No. 125	No. 126	No. 132	No. 138	Witte	Exp. No. 1	Exp. No. 2
Appearance	Granular, cream yellow powder	Fine, cream yellow powder	Fine, cream yellow powder	Coarse yellow powder	Fine, cream colored powder	Fine, brown powder	Fine, cream yellow powder	Fine, cream powder	Fine, cream powder
Odor	Slight putrid	Mild, not unpleasant	Rancid, unpleasant	Pleasant, sweetish	Mild, unpleasant	Burnt meat	Stale, unpleasant	Mild, not unpleasant	Mild, not unpleasant
Solubility (2%) in water	Readily in cold, colloidal turbidity Same on boiling	Readily in cold, slightly turbid solution. Ppt on boiling	Readily in cold, slightly turbid solution. Ppt on boiling	Readily in cold, slight turbidity. Ppt on boiling	Readily in cold, turbid solution. Ppt on boiling	Readily in cold, turbid solution. Same on boiling	Difficulty in cold, more easily on boiling. Heavy residue	Easily in cold, turbid solution. Moderate ppt. on boiling	Easily in cold, clear solution. Slight ppt. on boiling
Behavior in media preparation	Filters slowly	Filters readily. No difficulties	Filters readily. No difficulties	Filters readily	Filters readily. No difficulties	Filters with difficulty	Filters readily. No difficulties	Filters readily. No difficulties	Filters readily. No difficulties
Bouillon (appearance)	Dark colored turbid	Dark colored, clear	Light colored, clear	Dark colored, clear	Light colored, clear	Dark colored, turbid	Light colored, clear	Light colored, clear	Light colored, clear
Agar (appearance)	Dark colored clear	Dark colored, clear	Light colored, clear	Dark colored, clear	Light colored, clear	Dark colored, clear	Light colored, clear	Light colored, clear	Light colored, clear
Dunham's solution (appearance)	Slight turbidity, light yellow low	Clear, light yellow	Clear, light yellow	Clear, yellow	Clear, yellow	Clear, light yellow	Clear, almost colorless	Clear, almost colorless	Clear, almost colorless

TABLE II  
REACTIONS OF PEPTONE SAMPLES IN 1 PER CENT SOLUTION

## AMERICAN BRANDS

	No. 51	No. 67	No. 125	No. 126	No. 132	No. 138	Witte	Exp. No. 1	Exp. No. 2
Xanthoproteic	No ppt. with $\text{HNO}_3$ . Light yellow color. Orange + NaOH	No ppt. with $\text{HNO}_3$ . Light yellow color. Orange + NaOH	No ppt. with $\text{HNO}_3$ . Light yellow color. Orange + NaOH	No ppt. with $\text{HNO}_3$ . Light yellow color. Orange + NaOH	No ppt. with $\text{HNO}_3$ . Light yellow color. Orange + NaOH	No ppt. with $\text{HNO}_3$ . Light brown color. Orange + NaOH	No ppt. with $\text{HNO}_3$ . Deep yellow color. Orange + NaOH	No ppt. with $\text{HNO}_3$ . Low color. Orange + NaOH	No ppt. with $\text{HNO}_3$ . Yellow color. Orange + NaOH
Sat. Picric Acid Sol.	Moderate amorphous ppt.	Moderate amorphous ppt.	Slight ppt.	Slight ppt.	Slight ppt.	Slight ppt.	Heavy amorphous ppt.	Moderate amorphous ppt.	
Sat. Ammon. Sulph. Sol.	Slight ppt.	Slight ppt.	Trace of ppt.	Slight ppt.	Trace of ppt.	Slight ppt.	Heavy ppt.	Moderate ppt.	
Sat. Zinc Sulph. Sol.	Slight ppt.	Small ppt.	Trace of ppt.	Slight ppt.	Trace of ppt.	Trace of ppt.	Heavy ppt.	Moderate ppt.	
Millon's	Small ppt. Red on boiling	Heavy ppt. Deep red on boiling	Small ppt. Red on boiling	Moderate ppt. Red on boiling	Small ppt. Red on boiling	Heavy ppt. Deep red on boiling	Moderate ppt. Red on boiling	Heavy ppt. Deep red on boiling	
Biuret	No ppt. with $\text{CuSO}_4$ . Red color + KOH	No ppt. with $\text{CuSO}_4$ . Deep pink with KOH	No ppt. with $\text{CuSO}_4$ . Deep pink with KOH	No ppt. with $\text{CuSO}_4$ . Old rose with KOH	No ppt. with $\text{CuSO}_4$ . Purple pink + KOH	No ppt. with $\text{CuSO}_4$ . Pink + KOH	Small ppt. with $\text{CuSO}_4$ . Pink color + KOH	No ppt. with $\text{CuSO}_4$ . Pink + KOH	No ppt. with $\text{CuSO}_4$ . Deep pink + KOH
Glyoxylic. Hopkins-Cole	Strong ring	Small ring	Small ring	Strong ring	Very weak ring	Very weak ring	Strong ring	Very strong ring	Very strong ring
Sat. sod. Chloride Sol.	No ppt.	No ppt.	No ppt.	No ppt.	No ppt.	No ppt.	Slight ppt.	No ppt.	No ppt.
Pot. Ferrocyanide + Acetic Acid	Slight ppt.	No ppt.	No ppt.	No ppt.	No ppt.	No ppt.	Slight ppt.	No ppt.	No ppt.
95% Alcohol (3 vols.)	Slight ppt.	Trace of ppt.	Trace of ppt.	Trace of ppt.	Trace of ppt.	Trace of ppt.	Small ppt.	Small ppt.	Small ppt.
Sat. Picric Acid + NaOH.	Yellow color	Orange color	Yellow color	Red orange color	Slight orange color	Strong red orange color	Yellow color	Yellow color	Yellow color



*B. Laboratory Results*

Exclusive of experimental products, seven different brands of bacteriologic peptone were examined. These comprised samples of Witte's and six domestic products which are grouped in the succeeding tables of results under the caption of "American Brands." In addition to the preceding, there are included in the comparisons, two experimental products designated as "Exp. No. 1" and "Exp. No. 2," respectively. The first of these two peptones, after the basic constituents had been determined upon, was devised so as to give a product having as nearly as possible the same general reactions and gross chemical composition as Witte's peptone. Further work on bacterial utilization of peptones showing among other factors, greater degrees of hydrolysis than experimental No. 1, led to the preparation of the second of the two experimental products given in the tables. In all cases, the results tabulated in the foregoing are based on at least three separate samples of each brand, duplicate determinations having been made with each sample.

The physical examination, as may be noted from Table I, shows the general appearance of the various brands to be nearly the same. Only two products (No. 51 and No. 125) evidenced a decidedly unpleasant odor, the odor in one case (No. 51) being apparently due to microbial decomposition, as the moisture content of this sample was high.

A marked difference of solubility in water (2 per cent solution) is found between Witte's peptone and all the other brands examined. While the former dissolves only with difficulty in cold water and leaves a bulky residue on boiling, all of the other products readily go into solution in the cold, and leave only a small precipitate on boiling. Results obtained with experimental samples indicate that this residue given by Witte's peptone is partly due to its higher content of proteoses and other protein degradation products. In two cases (No. 51 and No. 138) the precipitate obtained was colloidal in nature, filtered with difficulty, and gave turbid bouillon and Dunham's solution, but had no effect on the agar. Media prepared from four of the samples (Nos. 51, 67, 126, and 138) were considerably more darkened than would be expected as a result of autoclave sterilization.

Table II shows that all of the brands examined give a strong xanthoproteic reaction indicating presence of tyrosin which is confirmed by the behavior with Millon's reagent. Tryptophane is shown by a strong Hopkins-Cole (Adamkiewicz) reaction in Witte's peptone, both experimental products, No. 51, and No. 126. Creatinine, as given by Jaffe's reaction, is present in perceptible amount in No. 138, and to lesser extent in Nos. 67, 126, and 132. Excluding the first experimental product which was prepared so as to contain albumoses in quantity, Witte's peptone is the only product showing an appreciable portion of albumoses as measured by the reactions with saturated ammonium sulphate, zinc sulphate and picric acid solutions. A small amount of primary proteoses in Witte's peptone is revealed by the precipitates obtained with copper sulphate (biuret test) and on addition of potassium ferrocyanide solution to an acetic acidified solution of the peptone. That this substance is not present in appreciable quantity, is shown by the absence of precipitate with the saturated sodium chloride reagent.

Both the biuret reaction and the behavior towards three volumes of 95 per cent alcohol were used as comparative tests to determine similarity with Witte's peptone rather than to detect any specific substance. Although the "biuret" color obtained with most of the samples, when the reaction was performed under uniform conditions, might be characterized as pink, there was still a considerable difference in the intensity of this color. From experimental preparations, it appears possible that the color factor is influenced by the degree and manner of hydrolizing the constituent proteins. All of the American brands appear to be nearly completely soluble in three volumes of 95 per cent alcohol, while both Witte's and the experimental products show small precipitates with this reagent.

TABLE III  
ANALYSES OF PEPTONE SAMPLES

	AMERICAN BRANDS							Exp.	Exp.
	No. 51	No. 67	No. 125	No. 126	No. 132	No. 138	Witte	No. 1	No. 2
Total Nitrogen	10.91%	13.50%	13.78%	12.41%	15.56%	12.42%	14.52%	14.35%	14.25%
Moisture	8.84%	5.52%	5.08%	10.20%	4.82%	6.43%	6.35%	5.13%	4.3%
Ash	13.71%	8.20%	3.98%	13.01%	3.92%	10.13%	3.44%	4.06%	2.86%
Phosphoric Acid as $P_2O_5$ in ash	0.18%	0.96%	1.66%	1.64%	1.05%	2.11%	0.42%	0.75%	1.02%
Calcium as CaO in ash	0.80%	0.45%	0.82%	0.52%	0.51%	0.70%	1.08%	1.13%	0.10%
Chlorides as NaCl in ash	7.76%	3.72%	0.13%	5.67%	0.26%	2.39%	0.54%	0.72%	0.35%
Reaction (1% sol.)	+9	+13	+15	+14	+11	+23	+6	+10	+13
Fulmer Scale									
H-ion	7.6x	5.0x	6.1x	5.8x	4.3x	8.0x	3.2x		6.8x
Conc'n (1% sol.)	$10^{-7}$	$10^{-6}$	$10^{-7}$	$10^{-7}$	$10^{-6}$	$10^{-6}$	$10^{-7}$		$10^{-7}$
Ratio Amino Acids (Witte = 1)	1.07	1.75	2.05	2.00	1.75	3.12	1.00		2.22

The analytical data presented in Table III, shows that not only is there a marked variation among the domestic products themselves, but also a difference from Witte's and the experimental products. The total nitrogen ranges from 10.91 per cent (No. 51) to 15.56 per cent (No. 132) with the "normal" as given by Witte's peptone, about 14.5 per cent. Moisture seems to be a more nearly constant factor, the average being about 6 per cent and only two samples (No. 51 and No. 126) show amounts above 8 per cent. These same samples also have the highest content of mineral matter, both over 13 per cent, and the lowest amount is shown by experimental No. 2 with less than 3 per cent. In all cases, the high ash content is found to consist mostly of chlorides, probably sodium chloride, and with one exception (No. 51), phosphates in addition. Calcium appears to be a predominant constituent in the ash of Witte's peptone and also

in the first of the experimental products. Most of the domestic samples appear to have this cation present in only about half the amount shown by the two brands mentioned.

Comparison of the acidity which 1 per cent aqueous solutions of the various brands give by the hot titration method shows Witte's peptone to have a value of +6 (Fuller Scale) against more than twice this amount recorded for nearly all of the other brands. The fallacy of "hot titration" and the errors in the use of phenolphthalein as an indicator for culture media reactions have been ably pointed out by Clark.<sup>15</sup> Determination of the actual hydrogen-ion concentration of these peptone solutions by the gas cell show three brands (Nos. 67, 132, and 138) to have what might be termed an appreciable acidity, above  $4 \times 10^{-6}$ , while the remainder are very nearly neutral. It is interesting to note, as substantiating the findings of Clark, that two of these three more acid peptones, Nos. 67, and 132, actually show *less* acidity by hot titration (+13, +11) than do peptones of smaller hydrogen-ion concentration, like No. 125, and No. 126, with values of +15 and +14, respectively (Fuller Scale).

The fact that Witte's peptone, as may be noted from Table II, is the least digested of any of the other brands examined, suggested the possibility that the acidity of a peptone solution when valued by hot titration is intimately associated with the amount of free amino acids present. Using Witte's peptone as a standard, colorimetric comparison of the amount of free amino acids in the various samples shows this to be the case. In other words, the high titration values are given by the products having higher amounts of free amino acids present. Aside from peptone No. 138 and Witte's, which represent the two extremes, the true reaction of the other products, as measured by their hydrogen-ion concentration, appears to bear no relationship to the free amino acids present.

In determining the nutritive value of peptones for general bacterial requirements, biologic tests are desirable which will give a definite, quantitative estimation. As a consequence, subculture transfers on slant agar in accordance with the method already outlined gave no satisfactory comparisons. The more delicate organisms, like the meningococcus, *M. catarrhalis* and *M. gonorrhæe* showed no viability on any peptone agar after the fifth or sixth transfer, while the more common semiparasitic types, which are not as complex in their food requirements, gave luxuriant growth with all of the peptones examined.

Attempts to value the peptone samples by agar plate estimations, as described under "Methods of Analysis," were abandoned. The organisms employed grew so luxuriantly in Dunham's solutions, made from all of the test peptones, that flocculent precipitates were formed which interfered with accurate counts by the plate method. Direct isolation of bacteria from pathologic material by use of media incorporating the test peptones appears to be a more promising index of nutritive value. This, however, is necessarily a slow process and comparative data to be used for this purpose is still accumulating. In view of the preceding, more significance was attached in valuing a sample to its utilization in the production of toxins and also, but to a lesser extent, in the formation of indol. It is appreciated that bacteriologists do not regard indol formation, *per se*, as of paramount importance, yet it will be conceded that a peptone which does not permit

of indol production would not be considered as practical for bacteriologic application. Aside from the practical importance of toxins in the production of anti-toxins and of indol formation in the sense just discussed, both of these products are capable of quantitative measurement.

TABLE IV  
BACTERIAL UTILIZATION OF SAMPLE PEPTONES

TEST PEPTONE	GROWTH B. DIPH- THERIE IN 2% BOUILLON	L + DOSE DIPH- THERIA TOXIN	GROWTH B. TETANI GLUCOSE BOUILLON	M. F. DOSE TETANUS TOXIN	GROWTH B. COLI IN DUNHAM'S SOL.	INDOL PRO- DUCTION B. COLI IN DUN- HAM'S SOL.
No. 51	Slow—Thin pellicle	Above 1.5 c.c.	Vigorous	0.33 c.c.	Heavy	Moderate
No. 67	Scant—No pellicle	Above 1.5 c.c.	Vigorous	0.25 c.c.	Heavy	Faint
No. 125	Moderate— Thin pellicle	Above 1.5 c.c.	Vigorous	0.25 c.c.	Heavy	Small
No. 126	Rapid and vigor- ous. Heavy pellicle	1.5 c.c.	Vigorous	0.25 c.c.	Heavy	Strong
No. 132	Scant—No pellicle	Above 1.5 c.c.	Vigorous	0.25 c.c.	Heavy	Faint
No. 138	Scant—No pellicle	Above 1.5 c.c.	Vigorous	0.25 c.c.	Heavy	Faint
Witte	Rapid and vigor- ous. Heavy pellicle	0.33 c.c.	Vigorous	0.25 c.c.	Moderate	Moderate
Exp. No. 1	Rapid and vigor- ous. Heavy pellicle	0.40 c.c.	Vigorous	0.25 c.c.	Heavy	Very strong
Exp. No. 2	Rapid and vigor- ous. Heavy pellicle	0.15 c.c.	Vigorous	0.25 c.c.	Heavy	Very strong

The results given in Table IV, show that only one of the domestic brands (No. 126) when incorporated into bouillon permits of vigorous growth and pellicle formation by the diphtheria bacillus. Luxuriant growth and heavy pellicle formation by Bact. diphtheriæ do not necessarily indicate strong toxin production as shown by the fact that the toxin in this case required 1.5 c.c. for an L + dose. Witte's peptone, as is known, also gives good growth, but a toxin of almost five times the strength (L + dose 0.33 c.c.). The most potent product, however, was furnished by "Experimental No. 2" with an average L + dose less than half that of Witte's (0.15 c.c.). Individual tests of toxins prepared with peptones made in a similar way have shown even higher toxicities (L + dose 0.10).

All of the products examined permit of vigorous growth and strong toxin formation with B. tetani. Tests with experimental preparations show that many hydrolized proteins have this capacity. As may be further noted from the table, heavy growth of B. coli was obtained with all of the products except Witte's when used in Dunham's media. With the Witte samples, the growth is moderate and diffused throughout the tube, and has no tendency toward precipitation as found with some of the other products. Corroborating the tests made for trypto-



phane given in Table II, the experimental products are found to give the strongest indol tests, with No. 126 next, while Witte's and No. 51 follow, both showing about the same intensity of color formation. Here again as with diphtheria toxin, luxuriance of growth is found to be no criterion of indol formation.

#### DISCUSSION

Consideration of the data presented in the preceding brings into question the value of the examinations, and the significance of the results obtained. In the final analysis, the value of any product must be gauged by the manner in which it fulfills *practical* requirements. With a substance like peptone, the point at issue is not whether it is in granular form or powdered, not whether it has a certain quantity of total nitrogen or chlorides, but how does it on practical test meet the needs of the bacteriologist. In other words, the product, to be of value, must contribute to the nutritive requirements of the ordinary saprophytic and parasitic organisms and yet be of sufficient flexibility in its constitution so as to furnish potent toxins or permit of tracing decomposition changes produced by an organism, as for example the elaboration of indol or the production of hydrogen sulphide. Illustrative of these versatility requirements is the fact that Witte's peptone is employed to advantage in the preparation of the complex media required for cultivating the spirochetes.

Based on these premises, the actual bacteriologic application of test peptones by incorporation in culture media is the crucial test of value. Aside from giving information as to the appearance of the media, the physical properties of a peptone are only of passing importance. Quantitative chemical analyses for the *gross* constituents are merely of value as means of controlling uniformity, once the general requirements for satisfactory peptone are established. Thus, a big deficiency in nitrogenous constituents as measured by total nitrogen, an actual, excessive acidity, or a large amount of mineral matter are undesirable factors revealed by such analyses. It must be borne in mind, in this connection, that the usual concentration in which the peptone is to be employed rarely exceeds 2 per cent.

More important information as to the applicability of a peptone for bacteriologic purposes is furnished by the qualitative examination for amino acids and other hydrolysis products. All protein bodies may be regarded as built up of a certain number of "bricks," of which amino acids play the most important part. Peptone, which is a protein degradation product actually has present a certain amount of amino acids in the free state, (Compare Table III), the amount depending on the method of preparation employed.

The value of the peptone in bacterial nutrition will be governed entirely by the "bricks" present which can be utilized by the bacteria. While some of these basic constituents are absolutely necessary for the maintenance of bacterial life and development, others in turn will be utilized, if present, for the production of certain, so to speak, "by-products," not essential to metabolism. Thus, as shown by the results given in Tables II and IV, the products containing appreciable quantities of tryptophane are found to give marked indol formation with *B. coli*. This, in a measure, also explains why *Bact. diphtheriae* can grow luxuriantly as in No. 126 without strong toxin formation. The results obtained in the study at

hand show, that among others tyrosin, as well as tryptophane, is an important constituent of a satisfactory bacteriologic peptone.

The bacteriologic data presented in Table IV show that the domestic products examined give either weak or no diphtheria toxin, while Witte's gives a decidedly potent product. On the basis of the above statements, this should be due to the presence of "bricks" in Witte's which are not present in the other brands. The validity of this hypothesis is shown by the fact that an experimental product (Experimental No. 1) devised so as to contain as nearly as possible the basic constituents of Witte's peptone and hydrolized to the same degree, gives very nearly the same results on practical, bacteriologic, and toxin tests.

From theoretical considerations the more simple the essential basic constituents are, the better will they be utilized by bacteria. Experimentation along this line has resulted in the preparation of a product (Experimental No. 2) which among other factors, has been hydrolized to a much greater degree than either Witte's peptone or the first experimental product. Table IV shows that where a measure of bacteriologic utilization is possible, as in production of diphtheria toxin, more than twice the potency is obtained. The results with other organisms, taking into consideration the limitations of such findings, fully substantiate the superior nutritive properties of the product.

#### SUMMARY

1. Physical properties and gross chemical analyses are of secondary importance in estimating the bacteriologic availability of peptones. More valuable information is given by determination of the protein hydrolysis products present.

2. Comparative values of practical importance are best furnished by certain biologic tests permitting of quantitative estimation. These are the elaboration of diphtheria and tetanus toxins, and to a lesser extent, the production of indol.

3. Domestic peptones furnish satisfactory tetanus toxin, but are unable to give a potent diphtheria toxin,—a property possessed by Witte's peptone. Indol producing power is shown by some of the domestic products as well as by Witte's peptone.

4. An experimental peptone is described which answers all the requirements of bacteriologic use, including the production of diphtheria and tetanus toxins, possessing superior activity.

#### BIBLIOGRAPHY

- <sup>1</sup>Bainbridge: Jour. Hyg., Cambridge, 1911, ii, 341.
- <sup>2</sup>Park and Williams: Jour. Exper. Med., 1896, i, 164.
- <sup>3</sup>Zipfel: Centralbl. f. Bakteriol., 1912, lxiv, 1. (Orig.) 65.
- <sup>4</sup>Cannon: Jour. Bacteriol., 1916, i, 536.
- <sup>5</sup>Standard Methods of Water Analysis A. P. H. A., 1912.
- <sup>6</sup>Methods of Analysis, A. O. A. C.—Bull. No. 107 (Rev.) Bur. of Chem. U. S. Dept. of Agr., 1912, 4.
- <sup>7</sup>Mohr: Cited in Treadwell-Hall, Analytical Chemistry, John Wiley & Sons, 1908, ii, 545.
- <sup>8</sup>Bömer: Ztschr. f. anal. Chem., 1895, v, 562.
- <sup>9</sup>Bigelow and Cook: Jour. Am. Chem. Soc., 1906, xxviii, 1496.
- <sup>10</sup>Bovic: Jour. Med. Research, 1915, xxxiii, 295.
- <sup>11</sup>Harding and MacLean: Jour. Biol. Chem., 1915, xx, 217.
- <sup>12</sup>Salkowski: Cited by Ford: Proc. Am. Pub. Health Assn., 1900, xxvi, 305.
- <sup>13</sup>Ehrlich: Cited by Böhme: Centralbl. f. Bakteriol., 1905, xl, 1 (Orig.), 129.
- <sup>14</sup>Rosenau: Bull. No. 21, Hyg. Lab. U. S. Pub. Health Service, 1915.
- <sup>15</sup>Rosenau and Anderson: Bull. No. 43, Hyg. Lab. U. S. Pub. Health Service, 1908.
- <sup>16</sup>Clark: Jour. Infect. Dis., 1915, xvii, 109.

# A TENTATIVE EXPLANATION OF THE MECHANISM OF HEMOLYSIS ASSOCIATED WITH LOSS OF WATER, AND THE BEARING OF THE PHENOMENON ON CERTAIN BIOLOGIC PROBLEMS\*

BY C. C. GUTHRIE, M.D., PH.D., PITTSBURGH, PA.

IT seems most probable that information leading to a better understanding of certain ultimate biologic processes will be achieved through the acquisition of more facts concerning the cell and its component parts. Naturally, questions concerning the morphologic and physicochemical properties of cell surfaces must be thoroughly understood before great progress can be made. For, the many-sided activities carried on between the interior cell substances and its surroundings must be understood before it is possible to unravel the ultimate questions concerned with intracellular metabolism. Therefore, hemolytic studies of any nature whatsoever should receive the most careful scrutiny and consideration from biologists representing the different fields in biology, in order to insure that observations of a fundamental character may not be overlooked. Other studies directed to the elucidation of facts concerning cell membrane properties using phenomena other than hemolysis, such as changes in irritability, reproduction, etc., are not of less importance, but in many instances the observations are subject to much greater error than is the case in hemolysis.

## CLASSIFICATION OF HEMOLYSIS

Agencies capable of producing hemolysis have been classified by Stewart<sup>1</sup> as follows: (1) mechanical means (pressure, trituration, shaking); (2) physical changes (freezing and thawing, heat, condenser discharges, water, drying and subsequent exposure to salt solutions); (3) chemical agents (saponin, bile salts, ether, chloroform, acids, alkalies, etc.); (4) biologic agents (the specific hemolysins—including the bodies active in putrefactive hemolysis, spontaneous laking, autolysis).

In regard to such classification, however, he says that it is "not a strict one, although it is useful for purposes of description. It is quite probable, for example, that interchange of water between the corpuscles and the suspending liquid is produced by the first group and that chemical changes are produced by the second. On the other hand, physical effects are unquestionably concerned in the action of such reagents as saponin, which dissolve cholesterol and lecithin. In biologic hemolysis, properly so-called, both chemical and physical reactions are also in all probability concerned."

The writer's interest in this subject is first indicated by a publication in 1903, entitled "The Laking of Dried Red Blood Corpuscles."<sup>2</sup> Though prior to that time the phenomenon had been observed,<sup>3</sup> nothing approaching a systematic study was found in the literature.

The action of a large number of reagents on the dried red corpuscles of man, the dog, cow, rabbit, chicken, and frog was studied.

\*From the Physiological Laboratory, University of Pittsburgh.



Blood films were spread on glass slides and allowed to dry, after which a cover slip was applied and the slide examined with the microscope. A drop of the reagent was then placed on the slide at the margin of the cover slip so that it would spread into the preparation, and the result was then observed.

Rapid laking was produced by addition of the animal's own, or the serum of another animal; or by the addition of iso- or hypertonic aqueous solutions of nonelectrolytes and electrolytes except such as readily convert the hemoglobin into insoluble substances; e. g., strong solutions of alcohol or of sodium hydroxide.

The precise amount of water which it was necessary to remove from the corpuscles in order to produce the alteration in the envelope, or stroma, on which the action depended, was not exactly determined; but experiment showed that it was necessary to remove some of the intracorpuseular water in addition to extracorpuseular water.

When gradual drying takes place in a wet blood mount, the corpuscles at the edges become enlarged, and extensive laking occurs.

Consideration of these results led to the belief that a better insight into the mechanisms involved might be obtained by studies of laking associated with loss of water by means other than evaporation, as by the application of hypertonic salt solutions, or by freezing and thawing.

#### HEMOLYSIS BY HYPERTONIC SOLUTIONS

With this in view, the hemolytic properties of hypertonic solutions of a number of relatively inert inorganic salts and other substances, including the chlorides of Na, K, Mg, Ca, and Ba; the sulphates of Na, K, Mg; cane sugar and glycerine were studied.<sup>4</sup> From the results it seemed that the degree of laking by hypertonic sodium chloride solutions or by hypertonic solutions of other inert salts depended upon the concentration of the solution; that in hypertonic solutions of inert substances in equimolecular concentrations, laking was not the same in all; that in equimolecular hypertonic concentrations, the chlorine salts were more powerful than the corresponding sulphates; and that nonelectrolytic solutions as cane sugar and glycerine, in hypertonic concentration produced laking, and this varied with the concentration of the solution.

At the time of publishing these results I was unaware that a few months previously Herzfeld<sup>5</sup> had published results of a similar study. He observed some difference in the resistance of corpuscles of different animals. Using 30 per cent NaCl he observed complete hemolysis of human blood in five minutes and of dog's in 20 to 25 minutes, and a greater resistance in rabbit's blood. Brahmachari,<sup>6</sup> who prior to Herzfeld had studied hemolysis by hypertonic solutions, also observed greater resistance of rabbit's blood to laking by strong sodium chloride solution.

#### HEMOLYSIS BY FREEZING AND THAWING

It was observed that blood could be repeatedly frozen and thawed, as in freezing point measurements, with slight or no laking. But when blood was exposed for some time to a temperature considerably below the freezing point, on thawing strong laking occurred.



Experiments were performed to determine what degree of cooling was necessary to cause laking, and to study the relation of the degree of laking to the length of time of exposure to such temperature.<sup>7</sup>

The blood of various animals including ox, dog, cat, and fowl were used. No marked differences in the behavior of the different bloods was observed. The results showed that slight or no laking occurred when the temperature of the blood was sufficiently lowered for the formation of crystals of ice if the blood was maintained at this temperature but a short time; that when maintained at a temperature between a point slightly lower than the freezing point and  $-1.0^{\circ}\text{C}$ . for ten minutes or more, laking occurred after room temperature had been restored for some time; and that the degree of laking, within limits, varied with the degree to which the temperature was lowered, and with the length of time the low temperature was maintained.

#### DISCUSSION

The mechanism of hemolysis by hypertonic solutions of salts of a neutral chemical character that do not readily penetrate cell membranes is obscure, though numerous possibilities exist and explanations have been offered. Brahmachari suggests that hypertonic solutions of sodium chloride lake by uniting with some cellular constituent and thus alter its normal properties. If this is true, it would seem that such union is at least of doubtful chemical character; for a number of inorganic salts as well as cane sugar and glycerine in hypertonic solution may cause laking.

From our knowledge of the morphology, chemistry, and the physicochemical relationships existing in cell membranes, it would seem that the action of salts in changing the physicochemical state of the proteins, as by precipitation or by a lesser change in the colloidal arrangement, may be an important factor. Lipoid and lipoid-like substances as lecithin and cholesterin are generally believed to be concerned in the formation of such membranes. Perhaps some type of union between these and other substances with protein constituents existing normally<sup>8</sup> are disrupted by salt action in such a manner that the normal architecture, so to speak, is irreparably injured. Since water is an ever present and essential constituent of all such structures, its withdrawal below a certain limit in itself may lead to or contribute to such a result. However this may be, certain phenomena observed in the cells under the influence of hypertonic solutions are difficult of explanation, as for example, fragmentation and swelling.<sup>9</sup> In the case of fragmentation, perhaps inequality of osmotic conditions or physical strength of the cell are factors. In the case of swelling or increase in size before laking, as may be observed under certain conditions, it is difficult to formulate a hypothesis. No doubt the reasons are physicochemical and in part due to change in permeability of the cell membrane. It would seem unlikely that a great change of the molecular concentration of the cell would occur under the conditions, yet this is indicated by the picture presented. It should not be overlooked, however, that the osmotic properties presented by living cells may be quite different from those present after death.<sup>10</sup> Therefore, toxic action of hemolytic agents, (toxic being used in the sense that the presence of substances presenting this quality is incompatible with

retention of cellular vitality due, for example, to production of asphyxiation), may be an important factor in concomitant lytic processes.

Neither is the mechanism of laking by freezing and thawing<sup>11</sup> understood. Since it is known that drying through evaporation will cause laking, it is possible that drying through crystallization of water<sup>12</sup> may account for laking by freezing. But again, since it is known that hypertonic solutions may cause laking, and since there is evidence that the freezing point of serum is somewhat higher than that of the intracellular liquids and, therefore, in freezing, a concentration of the serum occurs, it may be that such laking is fundamentally the same as laking by hypertonic solutions. Another consideration is that in thawing, water is liberated from the ice crystals and if the process be rapid, there is a possibility that uniform readmixture with the salts may not instantly take place—hence anisotonic conditions occur. The belief that frozen tissues have a better chance of surviving when slowly thawed points in this direction.

#### GENERAL CONSIDERATIONS

Such phenomena are of wide biologic interest. From this standpoint it is necessary to consider the effects of evaporation, hypertonic solutions and freezing and thawing upon multicellular organisms as a whole as well as upon individual cells.

A good example is the effect of evaporation upon frogs particularly in regard to body weight and function. Such studies gave very interesting results as regards the great loss of water that may rapidly take place without destruction of life; the very rapid taking up of water under such conditions; and the increase of the body weight.<sup>13</sup> Placed in wire cages in the air of the laboratory a very rapid loss of weight occurred, amounting in twenty-four hours to as much as 40 per cent to 45 per cent of the original weight. If the loss was not greater than this, recovery might take place if the animal were placed in water. The gain in weight was very rapid, the total weight within four hours amounting to as much as 121 per cent of the original weight, or 200 per cent of the weight after drying. Donaldson and Schoemaker<sup>14</sup> observed a great difference in the water content of frogs at different seasons. Durig<sup>15</sup> made more extensive observations upon the loss of water through evaporation and studied the effect upon body weight and upon tissue functions. In muscle-nerve studies on dried frogs, he in general observed decrease in the activities, which is in agreement with the statement of Davenport<sup>16</sup> in summing up the role of water that "it is essential to movement and those chemical processes which constitute metabolism."

It is a common experience in the physiologic laboratory to observe increased irritability of isolated nerves through evaporation. As a rule, hypertonic salt solutions irritate. A large number of substances both electrolytic and non-electrolytic that have the common property of being truly soluble, as salts of Na, K, NH<sub>4</sub>, Ca, Ba, Mg, and sugar and glycerine act in this manner.

Owing to the many statements made concerning a special inhibiting or anesthetic property of magnesium salts as compared to others, it is of interest that in our experiments this view has received no substantiation. Indeed, direct application of strong solutions of magnesium salts powerfully stimulate both sensory and

motor nerves, as shown by Liljestrand and others.<sup>17</sup> It is true that with enormous doses irritability is finally abolished, but this is true with many other salts. In fact, it is well known that anesthesia can be produced by water alone.

Lillie states that strong cytolytic action incidentally involves stimulation.<sup>18</sup> In the case of the electrolytes this may be due to the electrical charges associated with the ions acting upon the electrical state of the colloids, associated with solution and surface tension phenomena as described by Mathews.<sup>19</sup>

Many interesting observations have been made by experimental morphologists, zoologists and others, which serve to emphasize the very great importance of water content of tissues.<sup>20</sup> Of particular interest is the existence of forms which in nature are subjected to sudden periods of drought, such as rotifers. Definite protective mechanisms are found, such as the ability to encapsulate by means of a slimy secretion, which resist extreme desiccation.<sup>21</sup> An example of an interesting natural protective mechanism against low temperatures is furnished by the observation of Tower,<sup>22</sup> that before hibernation the potato beetle loses almost one-third of its weight of water, thus leading to concentration and lowering of the freezing point of its protoplasm.

Large differences are observed between the different classes of organisms in their abilities to resist and survive desiccation. The same is true regarding the ability of organisms to survive low temperatures. In the case of higher organisms an important consideration is the cessation of certain functions of specialized tissues at comparatively high temperatures after which general recovery is impossible. Different tissues of the same animal show marked differences in their susceptibility to low temperatures as judged by their recovery. For example, Cameron and Brownlee<sup>23</sup> found that recovery in frogs subjected to low temperatures may be prevented through injurious actions on the brain or cord. If the tissues of the entire animal are maintained for two hours at a temperature of  $-1.5^{\circ}$  and  $-.8^{\circ}$  C. recovery does not occur. Frog's heart muscle survives a temperature of  $-2.5^{\circ}$ , but is killed by a temperature of  $-3.0^{\circ}$ . Other muscular tissue will survive a temperature of  $-2.9^{\circ}$ , while peripheral nerves are not killed by much lower temperatures.

A very practical consideration connected with survival of animal tissues after freezing is the actual degree of temperature experienced by the cells themselves or intracellular components. A frog may be frozen until it is rigid and yet the cells will not be frozen, owing to the higher freezing point of body liquids, and also probably to the concentration of cellular contents associated with extracellular ice formation and to metabolic chemical processes continuing in the cells, resulting of course, in heat production. Also, there are reasons for believing that a cell does not freeze uniformly throughout. It has been shown<sup>24</sup> that the death temperature of the cell is lower than the point at which ice forms within it, also that intracellular oxidation is most active in the nucleus. Therefore, from the greater heat production it would tend to resist freezing more strongly than the cytoplasm.

#### SUMMARY

In each of the three methods of laking considered, a condition of hypertonicity or concentration of salts is present, but it is not possible to conclude that this

is the basic cause of the phenomenon in each case. It is believed, however, that sufficient evidence is available to render such a hypothesis tenable.

Hypertonicity may injure cells in many ways, but desiccation and change in physicochemical state are conspicuous possibilities in the studies presented. A few instances are cited to indicate the wide bearing of the problems involved.

## BIBLIOGRAPHY

- <sup>1</sup>Stewart: Jour. of Pharm. and Exper. Therap., 1909, i, 49.
- <sup>2</sup>Guthrie: Am. Jour. Physiol., 1903, viii, 441.
- <sup>3</sup>Stewart: Am. Jour. Physiol., 1902, viii, 117.
- <sup>4</sup>Guthrie and Lee: Proc. Soc. Exper. Biol. and Med., 1914, xi, 149.
- <sup>5</sup>Herzfeld: Ztschr. f. klin. Med., Berl., 1913, lxxviii, Nos. 5 and 6.  
See Bursy: Inaugural-Dissertation, Dorpat, 1863.
- <sup>6</sup>Brahmachari: Biochem. Jour., 1909, iv, 64.
- <sup>7</sup>Guthrie and Lee: Proc. Soc. Exper. Biol. and Med., 1914, xi, 150.
- <sup>8</sup>Matthews: Physiological Chemistry, 1915, 498; see also Bayliss: Principles of General Physiology, 1915, 127.
- <sup>9</sup>Hermann's Handbuch, 1880, iv, 13.
- <sup>10</sup>Matteucci: Univ. of Pisa Lectures, 1884; Am. Edition, 1848, 72; Also Reid: Jour. of Physiol., 1890, xi, 312.
- <sup>11</sup>Rollett: Sitzgeber. d. Wiener Acad., 1862, xlv, 65.
- <sup>12</sup>Müller-Thurgan: Landw. Jahrb., 1886, xv, 534.
- <sup>13</sup>Guthrie and Guthrie: Proc. Soc. Exper. Biol. and Med., 1914, xi, 144.
- <sup>14</sup>Donaldson and Schoemaker: Jour. Comp. Neurol., 1900, x, 109.
- <sup>15</sup>Durig: Arch. f. d. ges. Physiol., 1901, lxxxv, 401; 1901, lxxxvii, 43; xcii, 293.
- <sup>16</sup>Davenport: Experimental Morphology, 59.
- <sup>17</sup>Liljestrand: Skan. Arch. f. Physiol., 1909, xxii, 339.  
Guthrie and Ryan: Am. Jour. of Physiol., 1910, xxvi, 329.
- Guthrie and Lee: Proc. Soc. Exper. Biol. and Med., 1914, xi, 146.
- <sup>18</sup>Lillie: Am. Jour. of Physiol., 1912, xxix, 380.
- <sup>19</sup>Matthews: Physiological Chemistry, 1915, 228; Am. Jour. of Physiol., 1904, xi, 455.
- <sup>20</sup>Davenport: Loc. cit., 58.
- <sup>21</sup>Davis: '73, Monthly Micro. Jour., ix, 201-209, quoted by Davenport, loc. cit., 62.
- <sup>22</sup>Tower: Pub. Car. Inst. Wash., xlviii, 245, quoted by Cameron and Brownlee, Quar. Jour. of Exp. Physiol., 1913, vii, 120.
- <sup>23</sup>Cameron and Brownlee: Quar. Jour. of Exper. Physiol., 1913, vii, 115.
- <sup>24</sup>Apelt: Beitr. z. Biol. der Pflanzen, 1908, ix, 215; Zentralbl. f. Physiol., 1908, xxii, 538.  
Voigtländer: Unterkühlung u. Kältetod d. Pflanzen, 1910, ix, 359; Zentralbl. f. Physiol., 1910, xxiv, 271.



## STUDIES ON CHOLESTEROL\*

### III. INFLUENCE OF BILE DERIVATES IN BLOOR'S CHOLESTEROL DETERMINATION

BY GEORGINE LUDEN, M.D., ROCHESTER, MINN.

IN the latter part of 1915 Bloor<sup>3</sup> published his modification of the Autenrieth-Funk test for cholesterol in the blood. Shortly afterward he still further simplified his method by omitting the addition of sodium ethylate to the alcohol-ether extract of the blood before evaporation. Bloor's object in adding sodium ethylate had been: First, to destroy the "brownish tint," well known to all those who have worked on cholesterol; and second, to break up cholesterol esters by saponification.<sup>2</sup>

For the sake of brevity, the writer will refer to Bloor's original method as Bloor I, and to its subsequent modification as Bloor II.

Bloor himself has called attention to the fact that his method gives slightly higher values than that of Autenrieth, and he now attributes the brownish tint to overheating during the process of evaporation. An exhaustive study of the relative accuracy of the cholesterol values found by Autenrieth and the Bloor and digitonin methods has recently been published by Mueller.<sup>15</sup> Mueller had apparently based his work entirely on Bloor's second publication<sup>4</sup> as no mention of the first is found in his paper, though the effect of saponification on the cholesterol values is discussed in connection with experiments of his own. As regards the relative merits of the three methods mentioned he comes to the following conclusions: "Colorimetric analyses of the blood give results too high for true cholesterol because they include other ether- and chloroform-soluble substances, whereas digitonin determinations are the more nearly correct. Values obtained by colorimetric methods should be considered as representing cholesterol plus some more or less closely related substances, very likely of the nature of oxidation products." Mueller adds that there is some question as to the properties of these oxidation products, one of them possibly the so-called "oxycholesterol" that has been shown by the work of Lifschütz<sup>7, 8</sup> to exist in the blood normally, but that "since much of the oxycholesterol work remains as yet unconfirmed it is scarcely desirable to speculate on the exact part which is played by these derivatives in the results obtained in cholesterol determinations." As an answer to Mueller's comments on the Bloor method in its modified form, Bloor and Knudson published a new procedure for the "separate determination of cholesterol and cholesterol esters in small amounts of blood" by means of which they come to the conclusion that "either there are no other substances in blood plasma, or they behave, when treated with digitonin, in the same way as ordinary cholesterol."

In connection with these statements, a number of observations which I have made since May 15, 1916, on a series of 748 blood cholesterol determinations,\*\* may not be without interest. The series comprises 374 different blood samples

\*From the Mayo Clinic, Rochester, Minn.

\*\*This series does not include 400 samples examined before May 15, 1916.

including the blood of patients suffering from various diseases, specimens of my own blood in various experiments on nutrition, and weekly tests of the blood of experimental animals. In every instance parallel determinations of the same blood sample were made by the Bloor I and Bloor II methods; i. e., with and without the addition of sodium ethylate to the ether-alcohol extract before evaporation.

It was found that by the Bloor I test, lower values were obtained than by the Bloor II test in practically every instance, with the exception of a relatively small number of cases that will be discussed presently. In other words, the addition of sodium ethylate before evaporation seemed to destroy some color component which appeared to be responsible for the higher values found in the test without sodium. Mueller discusses the effect of strong alkali on the cholesterol values in relation to Autenreith's method, and shows by a number of tests devised by himself that "there is apparently no further loss after saponification." My experiments seem to show that the strong alkali may effect the color value of the test in a way in no wise related to the process of saponification.

It was further observed that the difference in the values obtained by the Bloor I and Bloor II methods was subject to slight variation in different blood samples, but that it ranged between 0.050 and 0.070 mg. in over 100 specimens of blood giving approximately "normal" cholesterol values.\* It was, therefore, decided to call this difference, provisionally at least, the "normal interval" between the two tests. On the other hand, a number of blood samples obtained from individuals of whose pathologic condition there could be little doubt, showed a marked deviation from this normal interval. The "pathologic" blood samples could be divided into two groups. One group, supplied by ten patients suffering from the same disease, gave identical values with both tests and will be discussed in another paper. The samples of the other group had been obtained from twenty patients with symptoms of disturbed liver function. The group included cases of severe obstructive jaundice, hemolytic jaundice, acute gastrointestinal catarrh (urobilin strongly positive in the urine) and various milder forms of "biliousness," with and without icterus. Every case belonging to the latter group showed a marked increase of the normal interval, ranging from 0.090 to 0.280 mg., the highest value being found in the blood of a patient who had been "extremely jaundiced since five weeks."

The deduction seemed admissible that the high values registered in these cases by the test without sodium might be due to the presence of bile derivatives in the blood, which could be partially or entirely eliminated by the sodium ethylate.

To verify this deduction a series of investigations was begun on Sept. 9, 1916, in order to determine: (1) whether any substance or group of substances belonging to the bile derivatives was capable of giving the Liebermann reaction in the definite absence of cholesterol, and (2) whether these bodies could be eliminated by the action of the sodium ethylate. Lifschütz<sup>7, 8</sup> states that bile acids do not give the Liebermann reaction, but it seemed possible that the combination

\*I find that my average normal cholesterol values are slightly higher than those of Bloor and Rothschild, ranging from 0.250 to 0.270 mg. This difference may be due partly to the personal equation inevitable in colorimetric determinations (personal color vision) and partly to the technic employed, which will be discussed further on.

of bile acids and bile pigments, a combination often found in icteric blood, might accelerate the process of oxidation, and the whole of Lifschütz' work suggests that oxidation plays an important part in Liebermann's cholesterol test.

The residue of gall stones from which the cholesterol had been thoroughly extracted (eleven successive extractions with boiling alcohol on the water-bath) seemed likely to contain the desired combination of bile acids and bile pigments and was therefore chosen as a starting point for these experiments. It consisted of a fine brown powder which dissolved fairly readily in pure chloroform, stained the chloroform deep canary yellow but also contained chloroform-insoluble elements that settled at the bottom of the flask in the form of a black, dust-like sediment. The latter was filtered off and dried on the filter paper at room temperature. Whereas the yellow chloroform solution might still contain traces of cholesterol, the black, chloroform-insoluble sediment could hardly be expected to contain any. However, since it was of paramount importance to eliminate every trace of cholesterol, the following procedure was adopted to "make assurance doubly sure:" Part of the black sediment was dissolved in ammonia water, filtered, and shaken out with pure chloroform in a separatory funnel. The chloroform was tested for cholesterol and remained colorless. Nevertheless, the operation was repeated three times as a measure of precaution. This may have been superfluous, but it will be remembered that the experiments were being made with a mixture of chemical compounds of unknown composition, present in unknown proportions, so that the established characteristics of any one single bile derivate could not be of any practical assistance in these experiments. Moreover the quantity of material at hand was very small; the entire yield of black sediment consisted of only 150 mg. Consequently every operation had to be a matter of "trying out things," with as little loss of material as possible. While the elimination of every trace of cholesterol remained the primary object, quantitative work was out of the question; hence the smallest possible amount of black sediment (as much as could be held on the point of a small scalpel) had been dissolved in the ammonia water.

After the third chloroform extraction the aqueous ammonia solution was acidified with hydrochloric acid, drop by drop (controlled with litmus paper) and this aqueous acid solution shaken out with fresh chloroform. The chloroform again assumed a vivid yellow tone and upon addition of the usual reagents (2 c.c. of acetic anhydrid, 0.1 c.c. of concentrated sulphuric acid to 6 c.c. of the chloroform solution) a Liebermann reaction of great color-intensity was obtained, such as is found only in "strong" cholesterol solutions (1 mg. in 1 c.c. of chloroform).

Bloor<sup>4</sup> (page 228) has called attention to the fact that it is difficult to extract traces of cholesterol from "strongly alkaline solution." It should here be stated that whereas the ammonia water used in this experiment was comparatively weak, lower concentrations gave less brilliant color reactions in the chloroform extract. This might be easily explained by the fact that the black sediment had not completely dissolved. On the other hand, the relative alkalinity of the solvent appeared to play an important part in the intensity of the color reaction, for even weak aqueous solutions of potassium or sodium hydroxide seemed to reduce the



color reaction of these bile derivatives in some unexplained way. Their chloroform extract remained colorless if it had been in contact with the alkaline solution for twelve hours, and gave no Liebermann reaction. Strong concentrations of ammonia water, however, only lessened the intensity of the reaction, and did not destroy it.

While these observations apparently confirmed the supposition that chloroform-soluble derivatives were unstable in the presence of alkali, they did not prove conclusively that sodium ethylate was responsible for their destruction in the Bloor I test, since in the latter the solubility of these bodies in ether-alcohol and, possibly, the effect of the heat during evaporation would have to be taken into account even if the presence of bile derivatives in the blood of icteric patients could reasonably be expected. Bile pigment has been demonstrated by Sahli.

The small amount of black sediment on hand, its unknown composition and the possibility that different portions of it might contain different mixtures of bile derivatives (which might in turn account for the varying results obtained with the different alkaline media) greatly complicated matters.

Finally, after many groping experiments, it was ascertained that ammonia water of a certain concentration (2 per cent) yielded the best results. Further trials with potassium and sodium had been given up on account of the loss of material they involved. The remaining 100 mg. of black sediment were dissolved in 100 c.c. of ammonia water of the above concentration, filtered, acidified with hydrochloric acid, and extracted repeatedly with pure chloroform until the latter became colorless and no longer gave a Liebermann reaction. The intensity of the reaction had been found to be proportional to the depth of yellow tone seen in the chloroform extract. The first extract was deep orange; the last had a faint yellow tinge. All the chloroform extractions were then mixed; the total amount of golden yellow chloroform thus obtained amounted to 450 c.c. The combined extracts were brought up to 500 c.c., giving a bile derivative solution that contained 0.2 mg. in 1 c.c. of chloroform.\* This solution (which for convenience had been termed "Pigment 28 solution") gave a brilliant color reaction, changing slowly from vivid pink to violet-blue and then to dark green. Six cubic centimeter tests were made with acetic anhydride and concentrated sulphuric acid in the usual proportions. The green stage of the reaction seemed to equal cholesterol reactions in depth of color, but differed slightly in tone. Whereas the shade of green seen in cholesterol solutions containing from 0.4 mg. to 10 mg. cholesterol per 6 c.c. of chloroform may be briefly defined as "emerald" green, all the writer's bile derivative solutions, the Pigment 28 solution included, were found to assume a slightly more yellow tone that might be compared to the color of an unripe olive. This olive green has also been observed by Luden in the cholesterol tests of pathologic blood samples and is probably identical with the slight brownish color commented on by Mueller,\*\* Bloor,<sup>4</sup> Autenrieth, and others.

\*The solution was made to obtain some idea of the relative color intensity of the Liebermann reaction which it gave. No quantitative accuracy could be aimed at since it would have been complicated by too many factors; i. e., the presence in the black sediment of a great number of chemical substances that might be precipitated in various degrees after acidification of the alkaline solution, and soluble to an equally varying extent in the chloroform. Consequently the solution may have contained slightly less than 0.2 mg. per cubic centimeter.

\*\*I am unable to agree with Mueller when he says that this "slight brownish color is invariably present in the chloroform solution of extracts prepared by Bloor's method." On the contrary, in as many as one-third of my determinations made by Bloor's method II, the chloroform solution was practically colorless and gave a brilliant emerald green reaction that could be matched quite easily with the standard solution. Tests made with Bloor's I method always gave a perfect match.



The color reaction described above must undoubtedly be considered a true Liebermann reaction, since it is produced by identical proportions of the same reagents commonly used in cholesterol determinations and since the order of the color sequence is identical with that described by Windaus<sup>20</sup> for cholesterol reactions.

Nevertheless, certain characteristic features exhibited by tests made with the chloroform solutions of the bile derivatives used in the writer's experiments, which distinguish *their* Liebermann reaction from that of pure cholesterol, would seem to make some special designation desirable. These features are: (1) The more olive green tone referred to above; (2) the brilliancy with which the *pink* and the *blue* stage of the reaction can be seen, even in weak solutions; (3) the length of time during which the last (green) stage of the reaction persists unchanged. Since these characteristic peculiarities are not found in Liebermann tests made with pure cholesterol-chloroform solutions, the term "bile-green reaction" in the Liebermann test is tentatively suggested; it will be used in this paper for the sake of brevity when the pink and blue stages of the reaction and its color persistency are discussed, and solutions containing the unidentified bile derivatives which give this reaction will be referred to as "Pigment solutions." The presence of biliverdin is uncertain in the black sediment from which the chloroform solutions had been made. Though biliverdin can be precipitated from alkaline solutions by hydrochloric acid, it is but little soluble in chloroform; consequently it can have been present only in traces in the chloroform solutions with which the writer made these tests. Bilirubin,<sup>16</sup> urobilin,<sup>17</sup> and cholic acid,<sup>21</sup> on the other hand, are alike soluble in aqueous alkaline solutions, precipitated by acidifying and readily soluble in chloroform. Since bilirubin, urobilin and cholic acid are found in gall stones, they were probably present in effective quantities in the Pigment 28 solution.

The following tests were made to illustrate the difference\* between the bile-green reaction and the reaction of pure cholesterol in the Liebermann test.

#### EXPERIMENTAL

(Tests showing that the pink and the blue stage of the Liebermann reaction can be seen in weak pigment solutions, but can not be observed in cholesterol solutions of a 400 times higher concentration.)

Windaus<sup>20</sup> states that the Liebermann reaction for cholesterol is characterized by a striking sequence of colors—vivid pink ("rosarot") blue, dark green—which may be observed as soon as the Liebermann reagents have been added to the test.

The writer was unable to find any trace of the pink and blue stages of the reaction in the cholesterol solutions commonly used for blood-cholesterol determinations; namely, solutions containing from 0.4 to 0.5 mg. cholesterol in 6 c.c.

\*Although this difference had been observed from the beginning and occurred in every solution made with chloroform extract of the black gall stone residue, these earlier tests (though their number includes several hundred observations) were not considered conclusive since the concentration of the solutions was unknown owing to the technical difficulties already described, and could not, therefore, be justly compared with the reaction in cholesterol tests of a known concentration. In every instance, however, these earlier observations were confirmed by tests made with the uniform solution of bile derivatives (containing approximately 0.2 mg. per 1 c.c. of chloroform) that has been described in detail under the name of "Pigment 28 solution."

of chloroform. In these the cholesterol appears to be oxidized almost immediately to the green stage of the reaction.

It seemed interesting, therefore, to ascertain how great the concentration of the cholesterol-chloroform solution would have to be—when the usual proportions were maintained—in order to show the sequence of colors described by Windaus.

TABLE I  
COLOR REACTIONS IN CHOLESTEROL SOLUTIONS\*

---

Test 1. 6 c.c. of the stock solution; namely, 6 mg. of cholesterol in 6 c.c. of chloroform: usual reagents added.
<i>Reaction:</i> The colorless solution becomes dark green in a few seconds; no trace of the pink or the blue stage.
Test 2. 10 mg. of cholesterol in 6 c.c. of chloroform: usual reagents added.
<i>Reaction:</i> Identical with Test 1, except that the green color appears almost black.
Test 3. 15 mg. of cholesterol in 6 c.c. of chloroform: usual reagents added.
<i>Reaction:</i> The blue stage appears for a few seconds only and is blotted out by the density of the green stage. The solution appears black; its green tone can be recognized only in a very strong light.
Test 4. 200 mg. of cholesterol dissolved in 6 c.c. of chloroform: usual reagents.
<i>Reaction:</i> As the sulphuric acid is added its progress towards the bottom of the test tube is shown by a streak of purple-violet, but the next second the whole of the solution has turned black-green. Its green color can be recognized only near the top edge by shaking the test tube.

---

Although the cholesterol concentration in these four tests exceeded that of the blood cholesterol from 10 to 400 times,† no trace of the pink stage of the Liebermann reaction could be seen in any of them. Nor could this stage be observed when one-half or one-quarter of the usual amount of the reagents had been used for a test. In either case the solution changed from colorless to green, though the intensity of the green color increased as time went on.

The results obtained with various dilutions of the pigment solutions showed a striking contrast to these cholesterol tests. The color sequence described by Windaus could be observed in solutions containing as little as 0.020 mg. bile green in 6 c.c. of chloroform.

To obtain a definite idea of the color value of these tests (previous observations had already suggested that their olive green tone made them appear darker than they really were) colorimetric determinations were made by the same method used in the blood cholesterol tests; namely, with the Duboscq colorimeter set at 10 mm. and a standard cholesterol test containing 0.4 mg. to every 6 c.c. of chloroform. An unexpected difficulty presented itself, however, due to the slow reaction of the pigment solutions, which had also been observed, but not fully realized. When parallel tests were made, such as are used in blood cholesterol

\*The standard cholesterol solution used in all of the writer's blood-cholesterol determinations contains 0.4 mg. of cholesterol in 6 c.c. of chloroform. It is made up from a stock solution containing 200 mg. of cholesterol (Merck) in 200 c.c. of chloroform.

Both standard and stock solutions are sealed with paraffine and kept in a refrigerator when not in use, in order to prevent any evaporation of the chloroform; the latter would increase their concentration, thereby affecting the accuracy of the tests.

Standardized graduated pipettes are used in all the tests in preference to graduated cylinders, since the latter often vary slightly and would give less accurate results in consequence.

†The highest cholesterol value found in a sample of pathologic blood was 0.720 mg. (Autenrieth method) but values of 0.450 (Bloor I) and 0.555 (Bloor II) have been seen repeatedly.

terminations, the cholesterol standard had begun to fade by the time the bile green reached its maximum color value.

A series of tests showed that the standard cholesterol test (0.4 mg. in 6 c.c. chloroform) reached its maximum in five to six minutes, maintained it for approximately thirty minutes at room temperature from 20° to 22°, had lost one-third of its original maximum color value in eighty minutes and had become colorless, that is, pale yellow, in six to seven hours. Pigment solutions of equal strength (0.4 mg. per 6 c.c. chloroform) on the contrary had not yet reached their maximum in one hundred and ninety minutes, and their color value remained unchanged for twenty-four hours, at least, under the same conditions.

Although blood cholesterol determinations are not complicated to the same extent by a different rate of reaction in the standard and the blood sample, *if the tests are made at room temperature*, (20° to 25° C.), and the standard test contains little more cholesterol than the blood specimen is likely to contain, the rapid reaction which occurs in some specimens of pathologic blood at a temperature of 35° to 37° would make it seem inadvisable to leave blood cholesterol tests at this relatively much higher temperature for a period of fifteen minutes according to the old method described by Autenrieth and Bloor. I have called attention to these facts and my recent findings appear to corroborate my observations.<sup>12</sup>

The period of time during which the standard cholesterol test gave a constant value at room temperature having been determined, a series of experiments was made with pigment solutions. A record was kept of the exact time at which the reagents were added to every test, in order to guard against even the slightest fading of the standard and to determine the length of the duration of the pink, blue, and green stages of the reaction in the pigment solutions. The results will be found in Table II.

In every instance the initial pink stage of the bile green reaction was clearly visible; the color value of the green stage was little over one-half of the cholesterol green in solutions of equal strength. Nonetheless, this green color was found to persist from three to four times as long as it did in the pure cholesterol solution.

That, owing to this slowness of the bile green reaction, this pink color is therefore bound to appear in blood cholesterol tests simultaneously with the green of the true cholesterol whenever any ether-alcohol and chloroform-soluble bile derivatives are present in the blood, may be deduced from the above. That the combination of the pink and green tones would account for the much-discussed brownish color will be readily granted.

Although the experiments described above seemed to furnish conclusive evidence that a true Liebermann reaction can be obtained in the chloroform solutions of some bile derivatives in the definite absence of cholesterol, several important points remained to be proved; namely, the elimination of these bile derivatives by sodium ethylate under conditions parallel to those found in the Bloor I method, and the close relation or identical characteristics of the bile derivatives obtained from cholesterol-free gall stone residue and those present in the blood of icteric patients.

The effect of the relative alkalinity of the aqueous solutions used as solvents for the gall stone residue had already suggested the instability of these bodies in

TABLE II  
COLOR REACTIONS IN PIGMENT SOLUTIONS

---

2 C.C. ACETIC ANHYDRID AND 0.1 CONCENTRATED SULPHURIC ACID ADDED TO EACH TEST. ROOM TEMPERATURE 20° TO 21° C.	
<hr/>	
Test 1. 1.2 mg. in 6 c.c. of chloroform. (Pigment 28 solution undiluted) (3 tests)	
Reaction:	The test solution turns bright reddish pink immediately. The pink color lasts for 20 minutes. The test tube is plunged in very hot water for a few seconds. The blue stage of the reaction appears, but has a dirty violet-blue tone. It is followed in 3 to 4 min by the green stage, intense olive green, which seems to be as dark as, if not darker than, cholesterol green of equal strength in the test tube.
Color value:	After 30 min. equal to 0.500 mg. cholesterol. (Standard cholesterol test* 0.400 mg.). After 6 hours equal to 0.666 mg. cholesterol. After 24 hours equal to 0.666 mg. cholesterol. After 48 hours equal to 0.666 mg. cholesterol. After 72 hours faded to a dirty brown.
Test 2. 0.400 mg. in 6 c.c. of chloroform. (5 tests.)	
Reaction:	The test solution turns bright, vivid pink immediately. The pink color lasts for 5 to 7 min. Dirty violet tone for 3 to 4 min. Olive green.
Color value:	After 20 min. equal to 0.180 mg. cholesterol. (Standard cholesterol test 0.400 mg.) After 60 min. equal to 0.200 mg. cholesterol. After 3 hours equal to 0.200 mg. cholesterol. After 24 hours equal to 0.222 mg. cholesterol. After 48 hours faded to a dirty brown. (Standard cholesterol test fades to dirty yellow in 6 hours.*)
Test 3. 0.120 mg. in 6 c.c. of chloroform. (3 tests.)	
Reaction:	Vivid pink for 5 min. Intermediate stage hard to define. Green in 10 min.
Color value:	After 30 min. equal to 0.060 mg. cholesterol. (Standard cholesterol test 0.400 mg.) After 3 hours equal to 0.056 mg. cholesterol. After 24 hours faded to a dirty yellow.
Test 4. 0.080 mg. in 6 c.c. of chloroform. (3 tests.) (Standard cholesterol test 0.400 mg.)	
Reaction:	Clear pale pink for 3 minutes. Intermediate color can not be recognized. Clear but light green in 6 to 8 minutes.
Color value:	After 30 min. equal to 0.050 mg. cholesterol. After 3 hours equal to 0.040 mg. cholesterol. After 24 hours faded to pale, dirty yellow.
Test 5. 0.040 mg. in 6 c.c. of chloroform. (3 tests.) (Standard cholesterol test 0.400 mg.)	
Reaction:	Clear pale pink; then pale green which lasts for about 3 hours.
Color value:	Can not be determined as test looks gray in colorimeter when compared with the emerald green standard, although its color is clear pale green in the test tube.
Test 6. 0.021 mg. in 6 c.c. of chloroform. (3 tests.) (Standard cholesterol test 0.400 mg.)	
Reaction:	Faint, but distinct pink; then very pale green lasting about 1 hour.
Color value:	Can not be determined for the same reasons as in Test 5. The green color is also clearly visible in the test tube.

---

the presence of strong alkalis, but the effect of heat during the process of evaporation no less than their relative solubility in ether-alcohol would have to be taken into account before definite conclusions could be reached.

In order to study the behavior of the bile derivatives contained in the Pigment 28 solution under conditions identical with those present in the Bloor I method, the following tests were made:

---

\*A newly made "ripe" standard was used for every determination, and for every test the colorimeter (Duboscq) was set at 10.0 mm.



Thirty cubic centimeters of the Pigment 28 solution were measured into a beaker and the chloroform removed by evaporation on the water-bath in order to avoid excessive heat at any one point in the solution. The orange-colored residue was extracted with 75 c.c. of Bloor's ether-alcohol mixture, brought to the boiling point on the water-bath, rapidly cooled under the tap to room temperature, filtered and made up to 100 c.c. with ether-alcohol. To one 20-c.c. portion of the ether-alcohol extract, sodium ethylate was added in the proportion recommended by Bloor (Bloor I), and the ether-alcohol evaporated, overheating being carefully avoided. Another 20-c.c. portion was merely evaporated, no sodium ethylate being added (Bloor II). The dry residue of both portions was extracted repeatedly with small quantities of pure chloroform, and the chloroform extracts made up to 12 c.c., respectively.

The Bloor I chloroform extract was colorless and remained colorless after the usual reagents (2 c.c. of acetic anhydrid, 0.1 c.c. concentrated sulphuric acid) had been added to a 6 c.c. portion of it, even when the test was warmed.

The Bloor II chloroform extract was golden yellow, and upon addition of the usual reagents, gave the brilliant variation of the Liebermann reaction already described.

The experiment was repeated four times with separate portions of the Pigment 28 solution and identical results were obtained in every test; i. e., the Bloor I tests remained colorless, while the Bloor II tests gave a vivid color reaction.

The fact that no color reaction could be obtained in the Bloor I samples suggested that a chloroform-insoluble sodium salt of the bile derivates might be formed by the use of the sodium ethylate and that the potassium salts of these substances might be more readily soluble in chloroform, especially in the presence of water, which would explain the brownish color in some of the Autenrieth tests and the results obtained by Mueller.\*

An opaque yellowish-white residue had been observed in the beakers of the Bloor I samples after the chloroform extraction. This residue was found to give the usual bile green reaction when dissolved in acid chloroform, but no color reaction could be obtained when the residue was extracted in the manner described for the pigment solutions although the residue itself dissolved readily in ammonia water. Many similar peculiarities that can not yet be accounted for nor discussed in detail at present have been observed in the writer's pigment solutions.

The destruction of the color reaction in Pigment 28 solution by the Bloor I method had, however, been proved, and colorimetric determinations were made to discover whether any loss occurred during the process of evaporation or subsequent filtration. This appeared to be the case as the Bloor II sample had lost approximately one-third of its original color value, and this may have been caused by further oxidation of some of the bile derivates. Since 30 c.c. of Pigment 28 solution containing 30 times 0.2 mg., or 6 mg., of bile derivates had been used, one chloroform test made with 10 c.c. of the ether-alcohol extract should have contained 0.6 mg. of bile derivates and should have given a higher color value than a test of a chloroform solution containing only 0.4 mg. of the bile derivates. The former test gave, however, a slightly lower reading than the

\*Mueller used potassium hydroxide 25 per cent in aqueous solution and 0.5 N. alcoholic potash.

latter; in other words, whereas a test containing 0.4 mg. of the bile derivatives had a color value equal to 0.222 mg. cholesterol, the test containing 0.6 mg. had a color value equivalent only to 0.200 mg. cholesterol.

Control tests made with solutions of pure cholesterol that had been treated in precisely the same manner described for the Pigment 28 solution by the Bloor I and Bloor II methods, gave color reactions of identical strength, a very slight loss of color value being, however, observed in both tests. Cholesterol Bloor I and Bloor II both registered 0.572 mg. when the original amount of cholesterol contained in the test had been 0.600 mg., and both tests registered 0.233 mg. when 0.240 mg. had been used originally. Tested against each other in the Duboscq colorimeter both tests gave identical values, whether the instrument was set at 10, 20, 30 or 40 mm. The slight loss referred to above may be technical.

That the sodium ethylate, and not the solubility of these bile derivatives in ether-alcohol, was responsible for the destruction of the color reaction in the Bloor I test was shown by the following: They dissolved equally readily in ether, in alcohol, in Bloor's ether-alcohol mixture, and also in petroleum ether.<sup>5</sup> If a few drops of sodium ethylate, however, were added either to a chloroform or to an ether-alcohol solution, the yellow color of the solvent became much lighter. But whereas the chloroform solution remained cloudy and a layer of yellow and brown precipitate could be seen at the bottom of the test tube, the ether-alcohol solution remained clear and contained but little of the bright yellow sediment (formation of a sodium salt of one or more of the bile derivatives?). It was further interesting that after evaporation of the ether-alcohol mixture the residue of the Bloor I (sodium) sample was opaque and yellowish-white, whereas the Bloor II was a transparent yellow. The same observation had been made in the dry residue of blood samples treated by both methods, with the exception that in blood extracts the Bloor II sample had a much fainter yellow color.

The destruction of the color reaction of the cholesterol-free gall stone residue by sodium ethylate having been established, the following questions remained to be answered: (1) Could the bile salts and bile pigments, whose presence might be reasonably assumed in the blood of icteric patients and has moreover been demonstrated by the work of Hoover and Blankenhorn be identical with those contained in the gall stone residue and their color reaction be similarly destroyed in the Bloor I Tests? (2) Could any means be found by which these bodies could be either identified with or differentiated from the oxysterol referred to by Mueller? My method of extraction (which is similar to that used for the recovery of bilirubin and cholic acid) suggested that the bodies contained in the Pigment 28 solution were not, in all probability, oxysterol, since the latter appears to be recovered together with cholesterol, and according to Matthews\* even Merck's "pure" cholesterol contains from 2 to 2.5 per cent of oxysterol. On the other hand, the presence of bilirubin and cholic acid, the latter dissociated from taurocholic acid by the acid or the alkaline solvents (Matthews,<sup>13</sup> pages 81-86) might be expected.

The method described by Lifschütz<sup>10</sup> to differentiate oxysterol from ox-

---

\*Unless the auto oxidation of cholesterol described by Schulze and Winterstein<sup>10</sup> alone should account for the presence of oxysterol in the Merck product.

dized cholic acid proved of the greatest value in answering both questions and also furnished a number of interesting observations that will be reported elsewhere, as they do not come within the scope of this paper.

Lifschütz states<sup>10</sup> that when either pure cholesterol, or pure cholic acid, or ordinary bile (he used the bile of oxen in his experiments) has been dissolved in glacial acetic acid and oxidized by means of a few particles of benzoyl peroxide, (the solutions being warmed gently to dissolve the peroxide, brought to the boiling point a couple of times and then rapidly cooled) either substance will give the same brilliant color reaction, changing from blue violet to deep green on the addition of 8 drops of sulphuric acid and 1 drop of ferri perchloride (Lifschütz' oxysterol reaction).

If, however, an equal volume of chloroform is added to the test after the reaction is completed and the mixture allowed to separate in a separatory funnel, the green color passes in its entirety into the *upper layer* when oxidized cholesterol has been used, the bottom layer appearing pale yellow or brownish, whereas in tests containing only oxidized cholic acid the upper layer is practically colorless and the *bottom* (chloroform) *layer* contains all the green color. (This test will be referred to as Lifschütz' "differential test.") If bile has been used for the reaction, both layers will be colored in direct proportion to the amount of either substance present in each layer, and the quantity can then be determined only by means of the spectroscope. According to Lifschütz, the green phase of the oxysterol is characterized by one single dark band at the red end of the spectrum, while spectroscopic examination of an oxidized cholic acid solution reveals four bands, a faint narrow band in the yellow, two broader and darker bands in the green (between the D and G lines) and practically total absorption of the violet.\*

In the absence of facilities for spectroscopic analysis, however, the differential test itself is sufficiently characteristic, according to Lifschütz<sup>10</sup> (page 394) to exclude all doubt concerning the identity of the bodies in question.

A series of differential tests was made by the Lifschütz method with the writer's Pigment 28 solution, pure cholesterol, and the extract of the blood of the highly icteric patient mentioned at the beginning of this paper.

For the purpose of making these tests it had been necessary to isolate the bodies that were responsible for the high value obtained by the Bloor II method in the blood extract. This had proved far more difficult than had been anticipated and more than thirty attempts had resulted in failure. At last, a simple method was found by which they could be secured by themselves in chloroform solution.

One hundred cubic centimeters of the patient's blood extract in ether-alcohol made with 6 c.c. of blood (that is, double the usual concentration for cholesterol tests) was slowly evaporated at room temperature. After twelve hours some 20 c.c. of the extract remained in the dish. This was poured off and the residue allowed to become completely dry. A black, slightly crustlike ring, 3 mm. wide and about 8 cm. in diameter, had formed where the liquid receded during evaporation. This ring dissolved very readily in ammonia water and the aqueous alkaline solution was filtered into a separatory funnel. The filter paper was then

\*Rosenheim's new color reaction for oxysterol has not yet been tried owing to some delay in obtaining the principal reagent (dimethylsulphate) and the great difficulties experienced in securing a reliable spectroscope.



washed with a weak solution of hydrochloric acid in water in order to dissolve any bile components that were more soluble in acid aqueous solution (since experience had shown such bodies to be among those found in the gall stone residue of unknown composition). The aqueous solution in the separatory funnel was acidified, and shaken out with a small quantity of chloroform.

Portions of this chloroform extract from icteric blood which had a slight yellow tinge similar to that found in weak pigment solutions, were used for the differential test. The chloroform was evaporated and the imperceptible residue dissolved in glacial acetic. Portions of the Pigment 28 solution were treated in the same way and small quantities of pure cholesterol (Merck) were also dissolved in glacial acetic to serve as control tests. Lifschütz mentions that the relative proportions given by him must be strictly observed in order to obtain the results he describes. These proportions have therefore been carefully maintained in all of the tests reported.

The results obtained with the solutions of pure cholesterol, bile green and the cholesterol-free bile derivatives of icteric blood in glacial acetic were as follows:

*Pure Cholesterol in Glacial Acetic Acid.*—After oxidation with benzoyl peroxide, boiling up and cooling, 8 drops of concentrated sulphuric acid and 1 drop of ferri perchloride were added. The solution turned deep burgundy red (first stage of oxysterol reaction) and changed from a purple blue to deep green on the addition of the 1 drop of ferri perchloride in glacial acetic acid. An equal volume of pure chloroform was then added to the test and mixture allowed to separate in a separatory funnel. All of the dark green color could be seen in the top layer shortly afterward. The bottom layer had a pale pinkish brown tone. The relative position of the colors did not change even after the test had stood for several hours, but the green color of the oxysterol gradually became a dark dirty brown (terminal stage of the reaction), while the lower stratum retained its original pink-brown tone. The same results could be obtained when test tubes were used instead of a separatory funnel.

*Bile Green in Glacial Acetic Acid.*—In this solution the reaction proved to be far more rapid than in the case of pure cholesterol. Mere boiling with benzoyl peroxide caused the test to assume the brilliant green tone peculiar to the third stage of the reaction. When sulphuric acid, 8 drops, and ferri perchloride 1 drop, were added, the green color darkened visibly and changed to a dirty olive brown in a few seconds (terminal stage of the reaction). On addition of chloroform in the separatory funnel this olive brown tone was found in its entirety in the bottom layer; the upper stratum remained colorless in the manner described by Lifschütz for solutions containing only cholic acid. This seemed to indicate that the bile derivatives found in the Pigment 28 solution were composed of, or closely related to, cholic acid, but that they contained no oxysterol. If the glacial acetic solution was mixed with chloroform as soon as the green stage of the reaction had been reached by boiling with benzoyl peroxide alone (no sulphuric or ferri perchloride being added) the intensely green color promptly settled in the bottom layer according to the behavior of cholic acid, and the upper layer again remained colorless.

The Pigment 28 residue had dissolved in glacial acetic with a golden yellow



color. If a few drops of concentrated sulphuric acid were added to the cold (golden yellow) solution, the first (burgundy red) stage of the reaction appeared immediately. If a portion of the golden yellow solution was left standing overnight at room temperature in an uncovered beaker, and therefore exposed to the action of the atmospheric oxygen, it was bright green in the morning, though traces of the red stage could be seen here and there at the bottom of the beaker.

It was also found that in Lifschütz' differential test the red stage of the reaction differs slightly from the green stage in its behavior towards chloroform. The red color goes into the chloroform practically in its entirety, but the upper layer has a very faint pink tinge. As soon as the green stage is reached, however, the upper layer becomes colorless, whereas the bottom layer is seen to contain all the color. In oxysterol solutions, on the contrary, the pink, the blue, the green and the terminal brown are all to be found in the upper stratum. This could be proved by adding sulphuric acid drop by drop and the addition of chloroform as soon as the test reached one of the first two stages.

The green stage of the reaction in oxidized cholic acid remained unchanged for days whereas the oxysterol green faded to dirty brown in about twelve hours. This peculiarity alone might suffice to distinguish oxidized cholic acid from oxysterol.\* It is, moreover, in accordance with the color persistency of my pigment solutions in the Liebermann test, and with Lifschütz' statement\*\* in relation to cholic acid: "Farbe wie Spectra halten sich wochenlang."

*Bile Derivates from Icteric Blood in Glacial Acetic Acid.*—After oxidation with benzoyl peroxide, boiling and cooling, the usual addition of concentrated sulphuric acid, 8 drops, and ferri perchloride, 1 drop, the glacial acetic solution of bile derivates from icteric blood turned bright yellow green, the third stage of the reaction being reached immediately because the amount of bile derivates contained in the test was relatively very small compared with the quantity of the reagents used. Chloroform having been added and separation having taken place, the upper layer remained colorless, showing that no oxysterol was contained in the solution. The bottom layer consisted of two strata, the lower, narrower stratum being dark green, the other bright yellow with a green tinge. This showed that the bile derivates extracted from icteric blood did not contain oxidized cholic acid alone but another substance as well. That this substance was not oxysterol was proved by the fact that it settled in the bottom layer and must consequently be closely allied to oxidized cholic acid. (The possibility suggests itself that it may have been the "rhizocholic" acid mentioned by Matthews<sup>13</sup> (page 340). Had any oxysterol been present, the upper layer could not have remained colorless, but both layers would have been colored in different degrees (Lifschütz).<sup>10</sup>

Without oxidation by benzoyl peroxide the glacial acetic solution of these bile derivates did not give Lifschütz' "Eisenchlorid-Schwefelsäure Reaktion,"

\*A specimen of pigment solution at the green stage of the Liebermann reaction now in my possession which was made and put into a separatory funnel on Sept. 11, 1916, is still bright green (Jan. 22, 1917), whereas the cholesterol test containing 200 mg. in 6 c.c. of chloroform became altogether a muddy brown in less than four days.

\*\*"(Es) ergibt sich die Frage ob die . . . mit grosser Leichtigkeit und Sicherheit hervorzurufende Reaktion auf Galle und Gallensäuren durch Oxydation derselben mit Benzoyl-superoxyd . . . sich nicht als Probe zur Ermittlung von Galle und Gallensäuren in den tierischen Flüssigkeiten eignen dürfte."<sup>9</sup> (page 326.)

even when the test was warmed. This is also in accordance with Lifschütz' statement "that cholic acid in glacial acetic remains colorless on addition of the reagents before oxidation."<sup>7</sup>

At the same time, however, in chloroform solution these cholesterol- and oxysterol-free bile derivatives were found to give the modified Liebermann reaction described for the writer's pigment solutions; that is, the pink stage of the reaction appeared with the vividness and brilliancy seen in high dilutions of bile green, but never observed in cholesterol solutions of the concentrations referred to in the beginning of this paper. Since Lifschütz states that pure oxidized cholic acid does not give the Liebermann reaction, the only explanation of its occurrence in the writer's solutions containing bile derivatives from icteric blood or from gall stones might be that the solutions contained a mixture of bile derivatives. That neither cholesterol nor oxysterol was present may be deduced from the methods used for extraction and from the results obtained with Lifschütz' differential test.

It seems possible, however, that the mixture of bile derivatives may have contained the "resin acid" described by Minovici,<sup>14</sup> which gives similar reactions and is the "B product" obtained by Minovici and Zenovici-Eremie after severe oxidation of cholesterol, but does not, according to these writers, contain any oxysterol.

Whatever the exact nature of these substances may be, their chemical peculiarities would seem well in accordance with Lifschütz' conclusion "that there can be little doubt concerning the gradual decomposition of cholesterol to cholic acid in the animal organism."<sup>\*</sup> Further investigations concerning the solubility of the bile salts formed in the Bloor I test and spectroscopic determinations will be reported when completed.

#### DISCUSSION

Although it is to be regretted that the observations made with Lifschütz' differential test could not be corroborated at present by spectroscopic analysis (owing to the scarcity of spectroscopes due to the war), the characteristic behavior of oxysterol and oxidized cholic acid, as well as the color persistency of the latter, would seem to leave little doubt as to the identity of the bodies contained in the writer's pigment solutions whether obtained from gall stone residue or from the cholesterol-free residue of icteric blood.

The curious fact that the glacial acetic solution of the bile green used in these tests should give the characteristic reaction of oxidized cholic acid immediately upon boiling with benzoyl peroxide and without the addition of concentrated sulphuric acid and ferri perchloride deserves a few words of comment. Lifschütz has shown that pure cholic acid gives this reaction only after severe oxidation, while the mixture of cholesterol-free bile derivatives from gall stones

<sup>\*</sup>"Die 'Nichtcholesterine' sind jedoch nachweislich Cholesterinabkömmlinge, da auch bei ihnen sich die Essigschwefelsäurereaktion des Oxycholesterins (durch Oxydation) hervorrufen lässt, und dienen sicherlich als Baumaterial für die N-freien Komponenten der Gallensäurepaarlinge."<sup>9</sup> (pages 327-328; 324.)

"Spinnt sich aber somit der Faden des Abbauprozesses vom Cholesterin bis zur Gallensäurebildung ab, so geht er sicherlich durch eine Reihe von Zwischengliedern und intermediäre Produkten hindurch."<sup>9</sup> (page 319.)

"Hieraus folgt der genetische Zusammenhang der Gallensäuren mit dem Cholesterin."<sup>9</sup> (page 303.)

gave the reaction when means were employed that would be insufficient to produce complete oxidation of pure cholic acid. This seems to indicate that the cholic acid which was present in these gall stone derivatives was already partially oxidized. Where this oxidation could have taken place is hard to understand, as the oxidizing power of the air oxygen during the process of extraction on the water-bath could hardly be compared to the effect of benzoyl peroxide, sulphuric acid and ferri perchloride combined, all of which are needed for the complete oxidation of pure cholic acid. Although pure (unoxidized) cholic acid could not be demonstrated in the mixture by the Mylius test, the presence of bilirubin could be shown by the test of Hammarsten. The question presents itself whether the presence of bilirubin could be in any way responsible for the partially oxidized condition of the cholic acid in the mixture. Bilirubin is known to be derived from hemoglobin (Matthews). Lifschütz<sup>11</sup> has demonstrated the strongly oxidizing properties of desiccated blood, by means of which he succeeded in oxidizing pure cholesterol (that contained no trace of oxysterol) into pure oxysterol which could be identified by its specific reaction and characteristic spectrum. It would, therefore, seem possible that some of the bile pigments present in the mixture had played a part in the partial oxidation of the cholic acid, no oxidizing agents having come into contact with the pigment solution until Lifschütz' differentiation test was made. The standard pigment solution used in all of the tests here reported had been kept well corked and in a cupboard where it was shielded from the light, as observations made on the ether-alcohol extracts of jaundiced blood seemed to indicate that the bodies which caused the excess color in the Bloor II tests were not insensible to light. A couple of blood samples that remained exposed to strong light seemed to give lower values with the non-sodium test after a few weeks. However, sufficient data have not yet been collected to warrant definite statements and further investigations are being made.

#### CONCLUSIONS

The results obtained in these experiments corroborate Mueller's statement that substances more or less closely related to cholesterol add to the values obtained in cholesterol determinations by Bloor's sodium-free modification which Mueller discusses. Lifschütz' differential test proved, moreover, that these substances are not oxysterol. Parallel determinations by both methods, Bloor I and Bloor II, seem to reveal the presence of bile derivatives in the blood which can be detected by means of colorimetric determinations and which it might not be easy to detect by other methods owing to the minute quantities in which they are present. Their study may nonetheless furnish valuable information concerning the chemistry of the blood in general.

Moreover, since colorimetric determinations and Bloor's method of extraction are comparatively simple and take little time, they are particularly suited for clinical observation. By parallel observations with the Bloor I and Bloor II methods, cases of disturbed liver function, for instance, might be conveniently singled out for further investigation with the Hoover-Blankenhorn<sup>6</sup> dialyzation method.



## SUMMARY

1. In 748 parallel blood-cholesterol determinations made according to Bloor's original method, with sodium ethylate, and Bloor's modification of his method, without sodium ethylate, a constant difference could be observed in the cholesterol values obtained by both methods, lower values being registered by the tests with sodium than by those without.

2. This difference was found to be most pronounced in samples of icteric blood obtained from cases of obstructive jaundice, but a constant slight difference, ranging from 0.050 mg. to 0.070 mg. could be observed also in normal blood.

3. A series of experiments showed that a mixture of cholesterol-free bile derivatives obtained from gall stone residue by a certain method of extraction gave a special type of Liebermann reaction in which the pink stage of the reaction could be seen in extremely high dilutions, whereas this pink stage can not be made visible in cholesterol solutions of equal or up to 400 times greater concentration.

4. It was found that this color reaction of cholesterol-free bile derivatives could be destroyed by the use of sodium ethylate under conditions parallel to those found in Bloor's original method, but that the color reaction of pure cholesterol could not be similarly destroyed. These observations seemed to account for the difference in the cholesterol values registered by tests made with Bloor's original method and by the sodium-free modification thereof.

5. The same modified Liebermann reaction could be obtained from the cholesterol-free residue of icteric blood.

6. That no oxysterol, but oxidized cholic acid, was present in the mixture of cholesterol-free bile derivatives whether obtained from gall stone residue or from icteric blood, could be proved by means of a test described by Lifschütz for the differentiation between oxysterol and oxidized cholic acid. In either case the behavior of these bile derivatives was identical with that of oxidized cholic acid as described by Lifschütz.

7. In one respect only did the cholesterol-free bile derivatives derived from gall stones differ from the derivatives of icteric blood; whereas the latter needed the complete oxidation required by cholic acid to give Lifschütz' differentiation test, the former gave this reaction with less vigorous oxidation.

8. Since Lifschütz has demonstrated the oxidizing properties of hemoglobin and since bilirubin, a hemoglobin derivative, could be demonstrated in the mixture of bile derivatives obtained from gall stones, it is tentatively suggested that the bilirubin contained in the mixture may have caused this partial oxidation of the gall stone derivatives.

9. My observations suggest that parallel cholesterol determinations with Bloor's original method and its modification might furnish valuable information concerning the chemistry of the blood, particularly in cases of biliary disturbance without visible symptoms of icterus, as Bloor's method is extremely simple and could easily be supplemented if desired by the dialyzation method of Hoover and Blankenhorn.



## BIBLIOGRAPHY

- <sup>1</sup>Autenrieth, W., and Funk, A.: Ueber kolorimetrische Bestimmungsmethoden: Die Bestimmung des Gesamtcholesterins in Blut und in den Organen, München. med. Wchnschr., 1913, i, 1243-1248.
- <sup>2</sup>Bloor, W. R.: Personal communication.
- <sup>3</sup>Bloor, W. R.: Studies on Blood Fat. II. Fat Absorption and the Blood Lipoids, Jour. Biol. Chem., 1915, xxiii, 317-326.
- <sup>4</sup>Bloor, W. R.: Determination of Cholesterol in the Blood, Jour. Biol. Chem., 1916, xxiv, 227-231.
- <sup>5</sup>Bloor, W. R., and Knudson, A.: The Separate Determination of Cholesterol and Cholesterol Esters in Small Amounts of Blood, Jour. Biol. Chem., 1916, xxvii, 107-112.
- <sup>6</sup>Hoover, C. F., and Blankenhorn, M. A.: Dissociated Jaundice, Arch. Int. Med., 1916, xviii, 289-303.
- <sup>7</sup>Lifschütz, J.: Die Oxydationsprodukte des Cholesterins in den tierischen Organen, III Mitteilung, Ztschr. f. physiol. Chem., 1908-1909, lviii, 175-184.
- <sup>8</sup>Lifschütz, J.: Die Oxydationsprodukte des Cholesterins in den tierischen Organen (Pfortader-Lebervene), Biochem. Ztschr., 1913, lii, 206-210.
- <sup>9</sup>Lifschütz, J.: Der Abbau des Cholesterins in den tierischen Organen (Studie), VI Mitteilung, Ztschr. f. physiol. Chem., 1914, xci, 309-328.
- <sup>10</sup>Lifschütz, J.: Die Abbauprodukte des Cholesterins in den tierischen Organen, VII Mitteilung, Ztschr. f. physiol. Chem., 1914, xcii, 384-401.
- <sup>11</sup>Lifschütz, J.: Die Oxidation des Cholesterins durch das Blutgewebe, VIII Mitteilung, Ztschr. f. physiol. Chem., 1914-1915, xciii, 209-227.
- <sup>12</sup>Luden, G.: Observations on the Changes in the Cholesterol Content of the Blood of Goats Following Cholesterin Feeding Alone, Roentgen Treatment Alone, and Cholesterin Feeding Combined with Roentgen Treatment and Subsequent Castration, Jour. Biol. Chem., 1916, xxvii, 273-295.
- <sup>13</sup>Matthews, A. P.: Physiological Chemistry, Wm. Wood & Co., New York, 1915.
- <sup>14</sup>Minovici, St., and Zenovici-Eremie, T.: Some New Oxidation Products of Cholesterol, Bull. sect. sci. acad. romaine, 1915, iv, 194-205; Abst. in Chem. Abstracts, 1916, x, 606-607.
- <sup>15</sup>Mueller, H. J.: A Comparison of the Results Obtained by Colorimetric and Gravimetric Determination of Cholesterol, Jour. Biol. Chem., 1916, xxv, 549-560.
- <sup>16</sup>Reinhold, B. von: Bilirubin, In Abderhalden, E.: Biochemisches Handlexikon, Berlin, 1911, Springer, vi, 278.
- <sup>17</sup>Reinhold, B. von: Urobilin, In Abderhalden, E.: Biochemisches Handlexikon, Berlin, 1911, Springer, vi, 288-290.
- <sup>18</sup>Rosenheim, M. C.: A New Color Reaction for "Oxycholesterol," Biochem. Jour., 1916, x, 176-182; Abst. in Chem. Abstracts, 1916, x, 3081.
- <sup>19</sup>Schulze, E., and Winterstein, E.: Ueber das Verhalten des Cholesterins gegen das Licht, Ztschr. f. physiol. Chem., 1904, xliii, 316-319. Cited by Lifschütz.
- <sup>20</sup>Windaus, A.: Cholesterin, In Abderhalden, E.: Biochemisches Handlexikon, Berlin, 1911, Springer, iii, 270.
- <sup>21</sup>Windaus, A.: Cholsäure (früher Cholasäure), In Abderhalden, E.: Biochemisches Handlexikon, Berlin, 1911, Springer, iii, 315.

## THE VALUE OF CALCIUM SULPHIDE IN THE TREATMENT OF POISONING BY MERCURIC CHLORIDE\*

BY CHARLES C. HASKELL AND R. H. COURTNEY, RICHMOND, VA.

POISONING with mercuric chloride, either intentional or accidental, has increased much in frequency in the past few years and the statement of Wilms<sup>1</sup> that in calcium sulphide we possess a valuable antidote is of much interest. Sabbatini<sup>2</sup> also experimented with sulphides, but his results were not so impressive. Lambert and Patterson<sup>3</sup> have shown that by means of forcing fluids and otherwise favoring rapidity of elimination, the average case of bichloride poisoning will recover with more or less severe symptoms. If, however, by means of a single intravenous injection of a solution of calcium sulphide we can be assured of the patient's recovery, a distinct advance has been made, for the tedious routine of Lambert and Patterson can be dispensed with and, of more importance, we have at our disposal a method which is certain in its results. This can be said of no other yet proposed.

The statement of Wilms' that local antidoting in the stomach is of little or no value is apparently borne out by the experiments of Burmeister and McNally<sup>4</sup> and of Fantus.<sup>5</sup> The first of these investigators showed that after oral administration of mercuric chloride, mercury is present in the blood in sufficient concentration within a very few minutes to cause renal damage; while Fantus has shown that the commonly recommended local antidotes exert little influence on the poisoning in rabbits following oral administration of mercuric chloride. If this is the case, it would seem better to adopt a method of administration that would insure completeness of absorption and not administer the mercuric chloride in tablet form orally, if we wish to determine the value of an antidote which will exert its influence on the mercury after absorption. Dogs are very prone to vomit after the oral administration of mercuric chloride and that in Wilms' experiments, conditions favorable for the production of emesis were present is indicated by the promptness with which this took place. It would seem probable that if emesis occurred within three minutes after the oral administration of a tablet which he "thought was the most insoluble tablet on the market" only a small amount of the mercuric chloride would be absorbed, barely more than the lethal dose; or, possibly not that amount, for only two of his three dogs used as controls died as the result of the mercury.

We have determined on dogs that recovery will usually occur after the subcutaneous dose of ten milligrams of mercuric chloride per kilogram of body weight, but that the dose of fifteen milligrams per kilogram is invariably fatal to untreated animals. This is illustrated by Table I.

If calcium sulphide is of value as an antidote for mercury after its entrance into the circulation, it should bring about the recovery of dogs that have received this surely lethal dose of mercuric chloride. We have carried out experiments

\*From the Laboratory of Pharmacology, Medical College of Virginia, Richmond, Va.

designed to answer this question by injecting relatively large doses of mercuric chloride and following this by intravenous injections of a solution of calcium sulphide.

The mercuric chloride was injected under the skin of the back in the form of a five per cent solution in distilled water. The calcium sulphide used was secured from two sources; one sample being manufactured by the Mallinckrodt Chemical Co., the other by the J. T. Baker Co. The solutions were made in either of the following ways. A gram of the calcium sulphide was added to about 450 grams of water in a flask and the solution boiled, to be then filtered into another flask and water added through filter until the weight of the contents was 456 grams; or a gram of the powder was added to the requisite amount of boiling water. As will be brought out later, the method of preparation is of importance. After filtration, the calcium sulphide solution was injected into the saphenous vein, exposed under cocaine anesthesia. Following Wilms' directions, the antidote was used "grain for grain" for the mercuric chloride injected. That the procedure exerted a beneficial influence is shown by Table II.

TABLE I

SEX	WEIGHT	DOSE IN MG. X KG.	RESULT	TIME
Female	18.45	15	Death	24 hours +
Female	5.90	15	"	24 hours +
Male	17.27	15	"	72 " +
Male	9.54	15	"	72 " +
Female	11.36	15	"	96 " +

TABLE II

WEIGHT	DOSE OF $\text{HgCl}_2$ IN MG. X KG.	MILS OF CAS SOL. X KG.	RESULT
7.38	20	9.16	Recovered
12.63	20	9.11	"
10.2	20	9.2	"

It is evident that the treatment with calcium sulphide solution was of benefit, for, as has been pointed out, the dose of 15 mg. per kilo is invariably fatal to untreated dogs. In all of these experiments, however, the injection of the calcium sulphide solution was made very soon after the injection of the solution of mercuric chloride, with one exception, where five hours intervened between the administration of the mercury and the antidote. As a rule, when a period of more than an hour has passed since the injection of the mercuric chloride solution, the results from the calcium sulphide are not so favorable, and the same is true if the dose be increased, as is shown in Table III:

TABLE III

WEIGHT	MG. $\text{HgCl}_2$ X KG.	TIME BETWEEN $\text{HgCl}_2$ AND CAS	RESULT
9.09	20	15 hours	Death in 48 hours
6.65	25	12 minutes	Death in 10 days
14.31	25	5 hours	Death in 48 hours +
5.36	25	5 hours and 23 min.	Death in 48 hours +
7.27	30	45 minutes	Death in 67 hours

It is evident, therefore, that the efficiency of calcium sulphide decreases with the lapse of time intervening between the administration of the mercury and the antidote and also with the size of the dose of mercuric chloride. In one experiment, a dog survived the dose of 30 mg. of mercuric chloride per kilo when the calcium sulphide solution was administered within fourteen minutes, but this may have been due to some error or to high resistance on the part of this particular dog.

Lambert and Patterson emphasize the importance of forcing fluid on patients poisoned by mercuric chloride, and at the time of the appearance of Wilms' paper, we were engaged on experiments, the object of which was to ascertain quantitatively, how valuable this procedure is. The details of these experiments will be published elsewhere, but it is of interest to compare the results secured by the intravenous injection of physiologic salt solution with those previously shown to have occurred after the use of calcium sulphide.

In these experiments, the same solution of mercuric chloride was used and the manner of injection was similar. The salt solution was made by dissolving technical sodium chloride in tap water and sterilizing by heat. There was from 0.9 to 1 per cent sodium chloride present, and the injections were made into the saphenous vein. For purposes of comparison, the calcium sulphide and the sodium chloride experiments are placed together in Table IV.

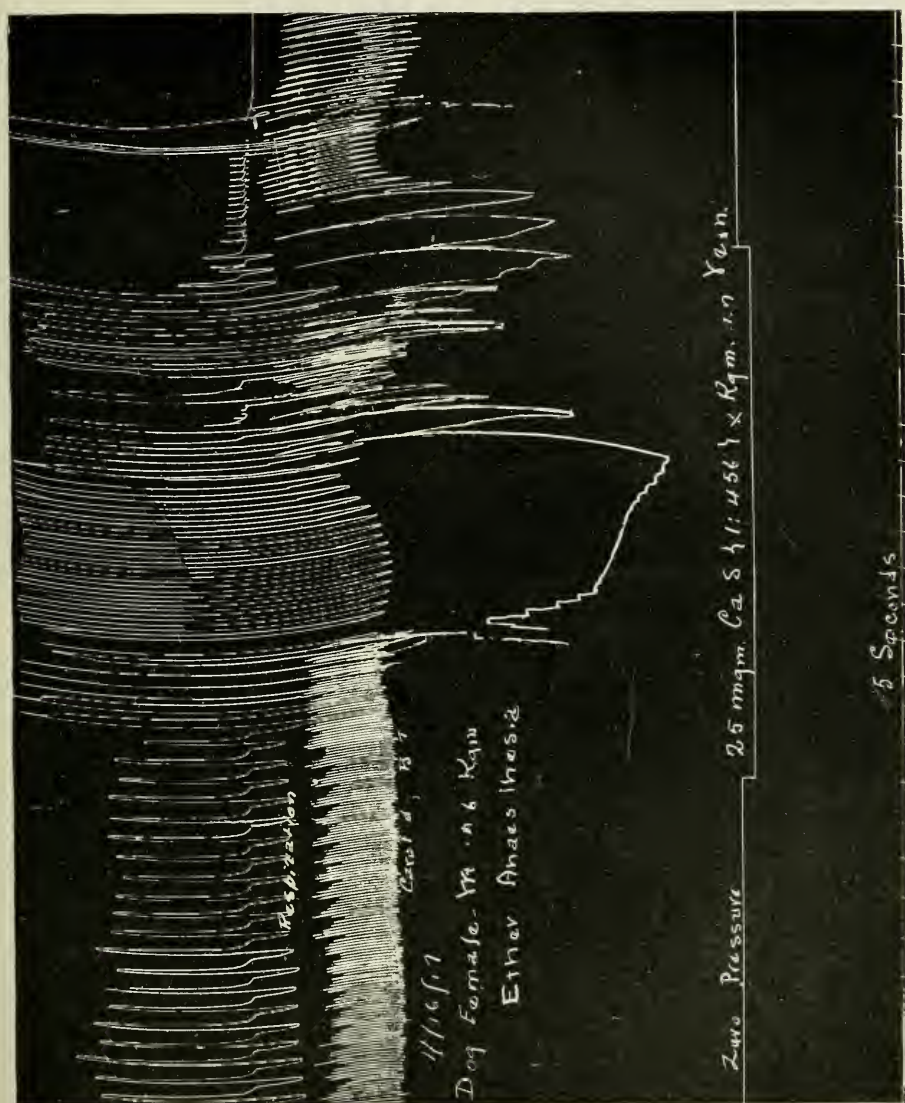
TABLE IV

WEIGHT	MGM. HGCL <sub>2</sub> PER KG.	ANTIDOTE IN MILS X KG.	TIME BETWEEN HG AND ANTIDOTE	RESULT
7.38	20	9 mils CaS Sol.	25 minutes	Recovery
12.63	20	Ditto	19 minutes	"
10.20	20	"	5 hours	"
11.10	20	9 mils Salt	48 minutes	"
6.81	20	"	5 hrs. 27 min.	Death sixth day
14.31	25	11 mils CaS	5 hours	Death in 48 hrs.
11.13	25	11 mils Salt	5 hours	Death on 6th day
5.36	25	11 mils CaS	5 hrs. 24 min.	Death in 48 hrs.
7.72	25	12 mils Salt	5 hrs. 36 min.	Death on 5th day

It would seem from this table that there is little to choose between the calcium sulphide solution and the saline. One dog recovered under calcium sulphide treatment when five hours had elapsed after the administration of 20 milligrams of mercuric chloride per kilo, while the sodium chloride control succumbed on the sixth day. To offset this, it is seen that with the larger doses of mercuric chloride, the sodium chloride dog lived longer in every instance than did the calcium sulphide animal. It must be remembered also that the Lambert-Patterson treatment depends upon the daily administration of fluid, colonic irrigation, and gastric lavage, the combination of which would undoubtedly give better results than a single intravenous injection of salt solution.

If, as seems to be the case, one is justified in assuming that the benefit of the calcium sulphide in the treatment of mercuric chloride poisoning after the poison has entered the circulation depends chiefly on the volume of fluid introduced, does calcium sulphide possess any advantage over physiologic salt solution? On





the contrary, we believe that salt solution is distinctly superior; because it is always readily secured, and, of much more importance, can cause no damage when used properly. The solution of calcium sulphide recommended by Wilms is, in the first place, decidedly hypotonic, and probably causes some hemolysis. In addition to this, Wilms points out that on allowing the solution to stand decomposition occurs, and substances are produced which are violently toxic. We have found that decomposition occurs also on boiling for a few minutes, and such a decomposed solution is capable of causing severe symptoms or even death if injected intravenously into a dog. In every experiment in which calcium sulphide was used, we have noticed the deep, gasping respiration mentioned by Wilms, and the report of the following experiment is particularly suggestive:

White and brown female fox terrier, weight 6.6 kilos.

May 16, 4.55 P.M.—25 mg. HgCl<sub>2</sub> x Kg. subcu.

5.07 P.M.—Started intravenous injection of solution of calcium sulphide which had been boiled 3 minutes. When about 5 mls x kg. had been injected, deep, gasping respiration was noted. Injection was continued, and respiration ceased entirely. Violent, tonic convulsion occurred, and artificial respiration was necessary for about five minutes. Animal was unable to stand for some ten or fifteen minutes.

May 26—Died during night.

To study more carefully the effects produced by these boiled solutions of calcium sulphide, records were taken of carotid blood-pressure and of respiration of two dogs under ether anesthesia. The tracing illustrates well the marked influence exerted by the intravenous injection of 25 milligrams of calcium sulphide per kilogram body weight. This solution had been made from the Mallinckrodt sample and was boiled for six minutes. The animal survived the effects of this injection, ether anesthesia seeming to increase the tolerance to the convulsant action of the decomposed solution.

While Wilms has suggested a means of treating poisoning by mercuric chloride which is certainly capable of causing the recovery of dogs that would, if untreated, succumb, nevertheless, we believe the following conclusions are justified:

1. The value of intravenous injections of solutions of calcium sulphide in the treatment of poisoning by mercuric chloride depends chiefly or entirely on the fluid which is introduced, and results fully as good can be secured by the intravenous injection of physiologic salt solution.

2. The intravenous injection of calcium sulphide is a procedure fraught with actual danger and it is possible that cases of mercurial poisoning may have the lethal exitus hastened rather than retarded by this administration of calcium sulphide.

3. In the present state of our knowledge, it would be unfortunate if we neglected to avail ourselves of every one of the measures that are of proved value in the treatment of bichloride poisoning; such as gastric lavage, colonic irrigation, hot packs, and the free exhibition of fluids, intravenously, subcutaneously, by rectum, and by mouth.

#### BIBLIOGRAPHY

- <sup>1</sup>Wilms, J. H.: Calcium Sulphide as the Chemical and Clinical Antidote for Mercuric Chloride Poisoning, etc., Jour. Lab. and Clin. Med., ii, No. 7, 445.
- <sup>2</sup>Sabbatini: L'acido solfidrico come antidoto generale del mercurio dal punto di vista fisico-chimico, Arch. Int. de Pharmacod., 1907, xvii, 319.
- <sup>3</sup>Lambert and Patterson: Poisoning by Mercuric Chloride and its Treatment, Arch. Int. Med., xvi, No. 5, 865.
- <sup>4</sup>Burmeister and McNally: Acute Mercury Poisoning, etc., Jour. Med. Research, xxxvi, No. 1, 87.
- <sup>5</sup>Fantus: Antidotes in Mercuric Chloride Poisoning, Jour. Lab. and Clin. Med., i, No. 12, 879.

## THE ETIOLOGY OF ARTERIOSCLEROSIS

BY LOUIS M. WARFIELD, A.B., M.D., MILWAUKEE, WIS.

ALTHOUGH a vast amount of work has been done in the attempt to produce arterial changes in animals which are comparable to those found in man in the group disease known as arteriosclerosis, the subject is still unsettled. As a matter of fact there is really no actual disease which we call arteriosclerosis. It is rather the end result in the arterial system of the action of several poisons elaborated in the body under a variety of conditions. So far as we now know these poisons may roughly be divided into two classes, (1) those produced by infectious diseases, and (2) those produced by the hypothetical, but probable, poison elaborated in the digestive tract as the result of the insufficient or aberrant digestion of the proteins. A third factor has undoubtedly a bearing on the production of arteriosclerosis in any individual. This is an indeterminate factor, it can not be controlled in the individual, for it is a part of the person's very birthright (or birthwrong), namely, heredity.

Still one other and definite factor is to be considered. This is syphilis. Studies of recent years leave no doubt that there is a specific form of arteritis caused by the spirochete pallida which may eventuate in a generalized arteriosclerosis.

An examination of the literature of the past few years reveals the fact that some investigators have produced arterial changes in small animals by the injection of various poisonous substances and blood pressure affecting substances, while others who have used these same substances have failed to confirm the results.

In 1908 D'Amato<sup>1</sup> reported some feeding experiments on dogs. He fed them for months on putrified meat and succeeded in producing inflammation and degeneration of the media and adventitia with calcification and hyperplasia of the intima. In the pulmonary artery, the carotids, in the venæ cavæ, and in the myocardium there were extensive necroses and hyaline degeneration. He also tried injections of sodium urate and ergot. These drugs caused necrosis in the muscularis and elastica of the aorta, pulmonary artery, vena cava inferior and heart muscle, but there was no subsequent calcification. His work showed that under quite grossly abnormal conditions of feeding, it was possible to produce arterial changes. One could scarcely affirm that he was able to produce arteriosclerosis analogous to that in humans.

Among others who have fed the ethereal esters of bacterial putrefaction to animals is Dratschinski<sup>2</sup> who fed indol for long periods in small doses to guinea pigs and monkeys. This apparently induced an atheromatous degeneration of the aorta and primary stage of sclerosis of the aorta and the vessels of the brain in the pigs. In monkeys sclerosis of the adrenals was produced.

Since Thayer and Brush<sup>3</sup> showed that the arteries of those who had had typhoid fever were more easily palpable than those persons of corresponding



age who had never had typhoid fever, attention has been directed to the relationship of infectious diseases and subsequent chronic arterial degenerative changes.

Frothingham,<sup>4</sup> for example, studied at autopsy the vessels of persons up to 40 years, who had died of infectious disease. It is interesting that he found demonstrable arterial lesions in those dying of infections or disease complicated with the pus-forming cocci. He also studied the arteries of rabbits suffering from experimental nephritis the arteries of a cat which had been glycosuric for over a year, and of rabbits injected with adrenaline and spartein. All these last revealed no arterial lesions. He concluded that although the study has thrown no light on the relation between noninfectious toxins and arterial disease, it has shown that during acute infectious diseases severe localized arterial lesions may occur.

Later he critically analyzed all claims made by those who said they had produced arteriosclerosis by the injection of various toxic substances or blood-pressure-raising substances in animals. He concluded that none has sufficient grounds for maintaining the successful production of arterial changes analogous to those which occur in man. Alcohol has been said by some to produce arteriosclerosis, by others it is said to have no effect.

It seemed that Longcope's work on the lesions produced by chronic protein intoxication should throw some light on the question, but he says\* that he was unable to find in his animals evidences of arteriosclerotic changes.

Further search through recent literature has not been rewarded by any more definite information.

The chronicity of the condition and the fact that it is the result of several diverse causes led to a clinical research among a group of inmates in the Milwaukee County Almshouse and Hospital. These results will be briefly described.

The material consisted of 500 persons ranging in age from 38 to 90 years. There were 412 men and 88 women. So far as possible a careful history was obtained with particular attention to the history and severity of various infectious diseases and pyogenic infections. The radial arteries were examined and note was made of the degree of palpability. Just palpable and judged to be sclerosis are called +; rather easily palpable are called ++; very easily palpable, beaded arteries were +++. At the same time the systolic and diastolic blood pressures were taken. As there was no relationship shown between blood pressure and palpable arteries of various grades, this feature has been omitted in the

TABLE I

DISEASE	NO.	+	++	+++	POSITIVE	NEGATIVE
Measles	47	10	6	12	28	19
Infectious arthritis	38	9	6	4	19	19
Pneumonia	30	5	8	5	18	12
Typhoid	27	6	8	3	17	10
Scarlet fever	10	0	0	4	4	6
Smallpox	14	1	4	0	5	9
Miscellaneous	12	2	5	2	9	3
	178	33	37	30	100	78

\*Personal communication.



study. Age had no influence so far as our studies were concerned. Old men of 80 had soft arteries while men of 40 had hard, palpable arteries, and no relationship could be shown between the infectious diseases and arteriosclerosis at different ages. I realize that in a study of this character there is much that may with justice be criticized, but certain rather interesting facts were brought out which seemed worthy of placing on record.

The cases were grouped according to history of single disease and those with two or more diseases in childhood or early adult life.

Measles heads the list with 47 cases, 10 were classed as +, 6 as ++, 12 as +++, total of 28. No palpable sclerosis was found in 19. Smallpox was the only disease in 14 cases. Of these one was +, four were ++, 9 were negative. (See Table I.)

When we come to examine all those with more or less severe single infectious diseases, we find 100 cases of sclerosis, 82 occurring in the severe infection group and 18 occurring in the mild group. There were 78 cases of definite sclerotic radials who had never had any infections.

There were 147 cases who had had one or more infections, but in whom there was no sclerosis made out. In 105 cases there was no history of infection and no evident sclerosis.

Of the 150 persons with multiple diseases, there were 80 whose radials were definitely found to be sclerotic, (37+, 35 ++, 8 +++). Seventy-two of these 80 had infectious diseases of severe nature, 8 had only mild attacks. Seventy showed no sclerosis. Forty-four of these had severe infections, 26 had mild infections.

Briefly these figures can be resolved into this table:

252 cases without sclerosis.
248 cases with sclerosis.
147 cases with infections but no sclerosis.
180 cases with infections and sclerosis.

#### SUMMARY

We confess that we began this investigation hoping to show that the infectious diseases played an important part in the production of arteriosclerosis. We are forced to conclude that they play little or no part. Possibly a study and tabulation of 5000 cases might lead to some more definite results. Certainly the very slight difference in favor of our supposition (from a study of 500 cases) is not great enough to be other than accidental.

#### BIBLIOGRAPHY

- <sup>1</sup>D'Amato: Virchows Arch. f. path. Anat., 1908, cxcii.
- <sup>2</sup>Dratschinski: Ann. de l'Inst. Pasteur, 1912, xxvi.
- <sup>3</sup>Thayer and Brush: Jour. Am. Med. Assn., 1904, xliii, 726.
- <sup>4</sup>Frothingham: Arch. Int. Med., 1911, viii, 153.
- <sup>5</sup>Frothingham: Bull. Johns Hopkins Hosp., 1913, xxiv, 323.

## LABORATORY METHODS

### A MODIFIED WASSERMANN TECHNIC BASED UPON THE RAPID FIXATION OF COMPLEMENT PRESENT IN HUMAN SERUM\*

BY C. J. BARTLETT AND A. L. O'SHANSKY, NEW HAVEN, CONN.

THE use of a complement-fixation test for syphilis in which the natural complement and the antishoop amboceptor in the patient's own serum are employed has become fairly common in controlling the Wassermann test. Probably the best known method based upon this principle is that introduced by Hecht<sup>1</sup> which was modified by Weinberg and still later further modified by Gradwohl.<sup>2</sup> This Hecht-Weinberg-Gradwohl method will, for the sake of brevity, be referred to as the Gradwohl method. After employing this Gradwohl method in a series of cases, a further modification was made by us by which it became evident that the natural complement of human blood serum combines with great readiness with the syphilitic antibody in the presence of lipoidal antigens. This union occurs much more readily than does that between the same complement and the antishoop amboceptor of the patient's serum in the presence of sheep corpuscles. In fact, this is so marked that if the proper quantity of the patient's serum is employed, the sheep corpuscles may be added to the tubes at the same time that the lipoidal antigen is added and then incubated without any previous period of complement fixation. The results are so constant that it appears that this technic may be employed to advantage to control the routine Wassermann test. As the patient's serum in this method is necessarily used without inactivating, there is no decrease of the syphilitic antibody as probably occurs in heating serum to 55° C. for 30 minutes; also as the serum is not incubated at 37° C. before the sheep corpuscles are added, there is no destruction of complement and only a minimum amount of serum need be used for the test. We have now employed this method in a series of 900 cases, paralleled by the routine Wassermann test, with satisfactory results.

It is desirable to describe briefly the technic of the Gradwohl method before giving that employed by us. As already indicated, the complement and the antishoop amboceptor of the patient's own serum are utilized in this test. Accordingly in using it there is first determined the amount of 5 per cent suspension of sheep corpuscles which is completely hemolyzed by .1 c.c. of the patient's serum and this amount is taken as the "hemolytic index" of that particular serum. From this index is then estimated the amount of the corpuscle suspension to be used in making the test. In most cases this represents only a fraction of the total

\*From the Pathological Society of the New Haven Hospital.

<sup>1</sup>Hecht, Hugo: *Wien. klin. Wchnschr.*, 1909, xxii, 338.

<sup>2</sup>Gradwohl, R. B. H.: *Jour. Am. Med. Assn.*, 1914, lxiii, 240; *Ibid.*, 1917, lxviii, 514.

amount of corpuscle suspension which .1 c.c. of the serum can hemolyze; for example, if this quantity of serum can hemolyze 1 c.c. of the 5 per cent suspension of sheep corpuscles, as found in determining the hemolytic index, only one-fifth of this amount or .2 c.c. is employed in carrying out the test. In making the test itself four tubes are used, the last one serving as a serum control and not receiving any antigen, while the other three receive increasing amounts of antigen. Each tube also receives .1 c.c. of the patient's serum and a sufficient saline solution to equalize the volume in each. They are kept in the water-bath at 37° C. for thirty minutes, after which the sheep corpuscle suspension in the amount computed from the hemolytic index is added to each tube and they are kept in the water-bath for thirty minutes longer. The reading is then made.

We have tried this method of Gradwohl in 250 cases controlled by the routine Wassermann test and also paralleled by the method about to be described, and it has not proved entirely satisfactory. In 14 per cent of the cases no hemolytic index could be obtained. In about 64 per cent, where an index was obtained, it was so low that according to Gradwohl's own statement, the results of the reaction must be regarded as of doubtful value. In this 64 per cent, and in the remaining 22 per cent where the index was higher, the results did not agree well enough with those of the routine Wassermann test to make the method appear to be of great value. Nor did it prove more sensitive than the regular Wassermann test. On the contrary, it gave a smaller number of positive tests. This appears to be due to the discrepancy between the amount of sheep corpuscles used for the test and the entire amount which the same quantity of serum used can hemolyze as shown in the hemolytic index; in other words, due to the use of an excessive quantity of either complement or amboceptor or both. However it is only fair to state that the only antigen used by us in making these tests by the Gradwohl method was an alcoholic extract of human heart, half saturated with cholesterolin.

The technic of the Gradwohl test for determining the hemolytic index was then modified by us by using a definite quantity of sheep corpuscle suspension in each tube and finding the smallest amount of the patient's serum which would produce complete hemolysis in 30 minutes in the water-bath at 37° C. The amount of sheep corpuscles determined upon was the same as that employed in our routine Wassermann test, .1 c.c. of a 5 per cent suspension in each tube, the entire quantity of material in the tube eventually being made up to .5 c.c. The technic is shown in Table I.

TABLE I.

FINDING THE HEMOLYTIC INDEX IN THE MODIFIED METHOD DESCRIBED.

Tubes.	1	2	3	4	5	6	7	8	9	10
Unheated serum.	.01	.02	.03	.04	.05	.06	.07	.08	.09	.10
5% sheep corpuscles.	.1 c.c. in each tube.									
85% salt solution	Up to .5 c.c.									

Incubate in the water-bath for 30 minutes at 37° C. Shake occasionally. Read index.

The tube containing the smallest amount of serum which shows complete hemolysis is taken as indicating the hemolytic index. Thus, if Tube 5 is the one

in which hemolysis is complete, the index is 5, indicating that .05 c.c. of that particular serum is sufficient to hemolyze .1 c.c. of 5 per cent suspension of sheep corpuscles. In about one-sixth of the sera in this series no hemolytic index was secured by this first test. In such cases a second series is set up similar to the above but having .11 c.c. of serum in the first tube and .01 more in each succeeding tube. In this series, if Tube 5 is the lowest one to show hemolysis, the hemolytic index is 15; that is, .15 c.c. of the serum is required for hemolysis. Over 80 per cent of the sera in this series, 731 out of 900, gave hemolytic indices between 1 and 10. Nearly 12 per cent (105) gave indices between 11 and 20; while 64, or 7.1 per cent gave no index, showing that .2 c.c. of each of these sera contain too little complement and amboceptor to completely hemolyze this quantity of sheep corpuscle suspension. It is to be noticed that the significance of a low hemolytic index by this technic is just the opposite from that in the Gradwohl method. Here a low index indicates that a serum is strongly hemolytic for sheep corpuscles while in the Gradwohl method, it shows the serum to have a low hemolyzing power.

Especial care must be exercised in determining the hemolytic index to make sure that the reading is sufficiently high. That is, if there is the least suspicion of any turbidity remaining in a tube when it is compared with one undoubtedly showing complete hemolysis, the one next higher in the series must be taken as showing the index. The importance of this is due to the fact that the exact amount of serum as determined by the index is to be employed in making the fixation test.

In obtaining the hemolytic index, our results do not agree with the statements usually made that the sera must be perfectly fresh in order to obtain the index. The 900 sera tested were, in the majority of cases, from twenty-four to forty-eight hours old, though the opportunity presented itself to secure them entirely fresh. At different times we have kept a serum for over a week and still secured an index, though at the end of this period this was somewhat higher than it was when the serum was fresh. The results obtained indicate that the serum, if kept aseptically and in an ice box, will give an index several days after it has been obtained, so that the test is applicable under the conditions ordinarily obtaining in making the routine Wassermann test in hospital laboratories. Presumably it will not prove applicable to sera which are sent from a distance so that they have been kept at room temperature for twenty-four hours or more.

The sera which gave no hemolytic index could not be tested by the method described without further modification. In nearly all of the cases here reported these sera were excluded as not being suitable for this test. More recently a method has been devised by which these also may be tested. This will be described later in this paper. The other sera, those giving a hemolytic index, were tested, after determining the index, as shown in Table II.

Each tube receives the serum to be tested and sheep corpuscles, and all except the first one, which is the serum control, also receive an antigen. It is to be recalled that this serum is not inactivated and, as shown in the table, is used in just sufficient quantity to completely hemolyze the corpuscles present, but with no excess. There are, therefore, in each of the last three tubes, complement, anti-



TABLE II.  
MODIFIED TEST, USING THREE ANTIGENS.

Tubes.	1	2	3	4
Unheated serum.		Indexed amount in each tube.		
5% sheep corpuscles.		0.1 c.c. in each tube.		
Antigen, diluted.	.0	0.2 c.c.	0.2 c.c.	0.2 c.c.
.85% saline solution.		Up to 0.5 c.c. in each tube.		

Keep in water-bath at 37° C. for 30 minutes with occasional shaking. Read the results.

The antigen used in Tube 2 was an alcoholic extract of human heart or guinea pig heart, half saturated with cholesterolin; in Tube 3, alcoholic extract of the same tissue; in Tube 4, an acetone insoluble antigen.

sheep amboceptor, antigen, sheep corpuscles, and, in cases of lues, syphilitic antibodies. The antigens must be strictly nonanticomplementary in the quantities used. As there is no period of heating at 37° C. before the corpuscles are added and so no appreciable lessening of the amount of complement present, it should follow that in all the control tubes and in all of the tubes where the complement is not very quickly fixed, hemolysis should occur. This result, we think, is shown by our tests. Also, that if hemolysis does not occur because of complement fixation in the syphilitic sera, the union, whatever its nature, between this natural complement, syphilitic antibody and lipoidal antigens must take place very much more rapidly than does that between the same complement, antsheep amboceptor and sheep corpuscles. This we believe to be a proper inference from our results.

Any serum which shows partial or complex lack of hemolysis in Tubes 2, 3, or 4 may then be tested by the scheme shown in Table III to determine the degree of inhibition. This test we have made with cholesterolized antigen only, as that is the most sensitive of the three antigens employed.

TABLE III.

MODIFIED WASSERMANN TEST, FINAL POSITIVE READING WITH CHOLESTERINIZED ANTIGEN.

Tubes.	1	2	3	4	5
Unheated serum.		Indexed amount in each tube.			
Antigen, diluted.	0	.2	.15	.1	.05
.85% saline solution.		Up to .5 c.c. in each tube.			

The reading is made as in the routine Wassermann test, complete inhibition in Tubes 2, 3, 4 and 5, with complete hemolysis in Tube 1, being + + + +.

Of the 900 sera which we have tested by this method, the first 231 were not carried through by the technic of Table II, but after determining the hemolytic index, they were tested only with a cholesterolized antigen as shown in Table III. The remainder were tested with three antigens and then the positive sera were tested as shown in Table III.

In several respects the technic as shown by Tables II and III closely parallel that of the routine Wassermann test as carried out by us. In this latter the total quantity in each tube is made up to .5 c.c. The amount of sheep corpuscles used in each tube is .1 c.c. of the 5 per cent suspension, and all sera are first tested with three antigens, using the same quantity as shown in Table II. All positive sera are then tested with the quantities of cholesterolized antigen as shown in Table III. The contrast between the modified test here described and the routine

Wassermann test is seen, first, in the employment of the complement and amboceptor of the patient's own serum in the former and, secondly, in the omitting in the modified test of any preliminary incubation at 37° C. for the purpose of complement fixation.

In comparing the results of the modified test described and the routine Wassermann test, we have obtained in the 900 sera 64 which gave no hemolytic index. Of the remaining 836, there were 629 which were negative by both tests, while 166 were positive by both tests. For the sake of comparison, all have been put down as positive which showed even slight inhibition of hemolysis. Thus, 795 agreed by both tests. Ten sera were positive with the routine Wassermann test and negative with the modified test described. Twenty-nine were positive with the modified test described and negative with the routine Wassermann test. In each of these cases a study of the history of the case was made when possible in order to determine which of the two tests gave the more reliable results. The summary of this study is given in Tables IV and V.

TABLE IV.

POSITIVE READINGS WITH THE ROUTINE WASSERMANN TEST AND NEGATIVE WITH THE MODIFIED TEST IN 900 SERA.\*

NO.	WASSERMANN TEST	MODIFIED WASSERMANN	HEMOLYTIC INDEX	SPECIFIC HISTORY
1	++±	0	6	Gastric ulcer, recurrent.
2	+++	0	6	Gastric ulcer, recurrent. Same as No. 1.
3	+±	0	6	Gastric ulcer, recurrent. Same as No. 1.
4	±	0	2	Brother of No. 1.
5	+++±	0	6	Pain in abdomen. Peritonitis.
6	±	0	12	Congenital lues. Wassermann 1 yr. ago, +.
7	++	0	10	Congenital lues. Other Wassermann tests ++ or +++.
8	++++	0	7	Husband said to have lues.
9	++++	0	5	Negative. Bichloride poisoning.
10	++++	0	6	Negative. Carbolic acid poisoning.**

\*For the sake of comparison, all tests showing even slight inhibition of hemolysis have been included in Tables IV, V, VI and VII.

\*\*One week later, both Wassermann and modified tests were negative.

In examining these tables it is noticeable that, of the cases in which the routine Wassermann test was positive and the modified test negative, only two gave a definite history of syphilis, one of these giving only a doubtful positive (±) and the other being ++. These were both cases of congenital lues. The husband of a third patient in this group was said to be syphilitic. Two of the sera giving a 4-positive were from cases of acute poisoning, and one of these, which was tested a week later, was then negative.

In Table V, twenty-nine sera which were positive with the modified test and negative by the routine Wassermann test came from twenty-six patients. Twelve of these gave a history of syphilis or were suffering from diseases like tabes or general paresis which are recognized as being of luetic origin. Several of the others gave a + or ±, a reaction too weak for a definite diagnosis, but they have been included here for the sake of comparison.

TABLE V.

POSITIVE READING WITH THE MODIFIED TEST AND NEGATIVE WITH THE ROUTINE WASSERMANN TEST IN 900 SERA.

NO.	WASSERMANN TEST	MODIFIED WASSERMANN	HEMOLYTIC INDEX	SPECIFIC HISTORY
1	0	++	4	Tabes. No history of primary.
2	0	++	5	Pulmonary stenosis.
3	0	+	6	Pulmonary stenosis. Same as No. 2.
4	0	++++	3	Congenital lues.
5	0	+++±	10	Primary, 1916.
6	0	+±	6	Hemiplegia.
7	0	+++±	3	Primary, 1916.
8	0	±	4	Loss of weight, pain in stomach.
9	0	+++±	4	Tabes. Spinal fluid, positive +±.
10	0	+±	6	Primary two years ago.
11	0	+++±	2	Gonorrheal arthritis, 1915.
12	0	++	4	Tertiary lues. Wassermann test, +++.
13	0	++++	4	Furunculosis.
14	0	++	10	General paresis.
15	0	+	4	Tabes.
16	0	+++±	4	Cerebral syphilis. Wassermann positive two years ago.
17	0	++	5	Negative. Eczema.
18	0	+±	10	Tabes.
19	0	++±	7	Negative. Hemiplegia.
20	0	++	5	Ulcers of leg.
21	0	±	4	Negative. Acute pleurisy.
22	0	±	4	Diagnosis is lues. Salvarsan treatment.
23	0	±	2	Negative. Furunculosis.
24	0	+	5	Negative. Arthritis.
25	0	+	12	No history obtained.
26	0	++++	12	Diagnosis, cerebellar tumor. Spinal fluid negative.
27	0	±	7	Same as 26. Made shortly after provocative salvarsan.
28	0	±	5	Wife has syphilis.
29	0	++±	7	Tabes. Same as No. 18. Spinal fluid +++.

In the first 500 sera tested, there were 31 from 30 different patients in which a positive result was obtained by each method but in which the modified method gave a higher reading than did the routine Wassermann, while in 9 sera the opposite was the case. These are given in Tables VI and VII.

Of the 31 sera where the modified test gave a higher reading, 22 from 21 different patients gave a history of syphilis or the symptoms were such that a diagnosis of luetic infection had been made. In addition, one other patient had had five abortions, and in a third the placenta had an appearance which was considered suspicious of syphilis, while a fourth had osteitis of the tibia. Included in the above 22 were three cases of tabes, four in which the diagnosis of cerebro-spinal syphilis had been made and one of congenital syphilis. The variation in the degree of complement fixation varied from ± by the routine Wassermann with +++++ by the modified method to ± by the former with + by the latter.

Of the 9 sera from as many different patients in which the routine Wassermann gave the higher reading, 6 gave a history indicative of syphilis. The great-

TABLE VI.

BOTH TESTS POSITIVE. MODIFIED METHOD GIVING THE HIGHER READING.\* 500 SERA.

NO.	WASSERMANN TEST	MODIFIED WASSERMANN TEST	HEMOLYTIC INDEX	SPECIFIC HISTORY
1	++	++++	7	Negative. Necrosis, superior maxilla.
2	±	++	5	Primary fourteen years ago.
3	±	++±	13	Primary, ten years ago.
4	++	++±	4	Negative. Infection, both hands.
5	±	+±	10	Secondaries present.
6	±	+	4	Cerebrospinal lues.
8	±	++±	4	Tabes. Salvarsan treatment.
7	±	++	7	Primary in 1911. Salvarsan treatment.
9	++±	+++±	15	Pneumonia.
10	±	+++±	3	Congenital lues.
11	±	++	5	Primary, three years ago.
12	++±	++++	3	Treatment for syphilis for two years.
13	±	+±	5	Five abortions.
14	±	++±	5	Pain in chest. Has had KI.
15	++±	+++±	7	Primary in 1915. Treated with mercury.
16	±	+++±	2	Paraplegia.
17	±	++++	2	Primary, twenty years ago. Furunculosis.
18	++±	++++	4	Tabes.
19	+±	+++	2	Same as No. 17.
20	+	++	5	Tabes.
21	±	+++±	10	Cerebral lues. Hemiplegia.
22	+±	+++	4	Treated for lues.
23	±	+++±	5	Primary in 1912. Salvarsan and mercury treatment.
24	+±	+++±	5	Hemiplegia.
25	+	+±	2	Placenta suspicious of syphilis.
26	++±	++++	10	Cerebrospinal lues.
27	±	+±	4	Two stillbirths.
28	±	++++	4	Gumma of rectum. Previous Wassermann, +++.
29	++±	++++	4	Osteitis tibiæ.
30	±	++++	5	Primary four years ago.
31	±	+±	6	Cerebrospinal lues.

TABLE VII.

BOTH TESTS POSITIVE. ROUTINE WASSERMANN METHOD GIVING THE HIGHER READING.  
500 SERA.

NO.	WASSERMANN TEST	MODIFIED WASSERMANN TEST	HEMOLYTIC INDEX	SPECIFIC HISTORY
1	+++	++	5	Primary twenty years ago.
2	++++	+++	2	Syphilis. Miscarriage.
3	++++	++	8	Epilepsy.
4	++++	+++±	7	Myocarditis. Emphysema.
5	++++	+++±	8	Primary six months ago.
6	++++	++	7	Pain in feet.
7	++++	+++±	11	Latent syphilis.
8	++++	+++±	3	No history of primary. Has been treated for syphilis.
9	+++±	++	12	Wassermann, positive (+) three years ago.



est variation in the two readings here was + + + + to + +. A careful study of these Tables, IV, V, VI and VII, in which there is a discrepancy between the two tests, leads us to feel that the modified test in this series is not only more delicate than the routine Wassermann test but also that it is more accurate; also that it does not give an undue number of false positive tests. We appreciate, however, that false positive results do occur at times, even when these are 4-positive. Thus, No. 26 of Table V, a case diagnosed as cerebellar tumor, gave 4-positive by this method and negative by the regular Wassermann, but after a provocative dose of salvarsan, it became only a doubtful positive by the modified method, and a little later was entirely negative. Another case, too recent to be included in the 900 reported, gave similar results, and is improving under tuberculin treatment.

Among the 900 sera there have been six which were anticomplementary by the routine Wassermann method, using inactivated sera. Of these, two also failed to give any hemolytic index with the modified method. Of the other four, one, a case of staphylococcus septicemia, gave a hemolytic index of four and was negative; one, a case of Banti's disease, gave an index of 6 and was negative; the third, carcinoma involving the bile ducts, gave an index of 6 and was negative; and the fourth, a case of congenital syphilis with treatment, with an index of 13, gave a weak positive.

The 64 sera, which gave no hemolytic index, came from a variety of conditions and did not appear to be particularly related to any one type of disease. In attempting to obtain the hemolytic index in samples of sera from placental blood and of spinal fluid, it was found that no hemolysis occurred. Either complement or amboceptor, or both were lacking. Until recently we have been unable to test these for the same reason that the other sera having no hemolytic index could not be tested. Recently we have found that by combining in equal quantities a negative serum having a comparatively low index, with a serum giving no index or with a spinal fluid, the combined serum or serum and spinal fluid, will give a hemolytic index and can be tested by the method described. We have as yet applied this to only a small number of sera having no hemolytic indices and to a small number of spinal fluids, but it apparently makes it possible to test any serum and any spinal fluid by this method. We have not had opportunity to apply it as yet to the serum from placental blood.

One evident criticism of the method as described is that the quantity of serum used in performing the test varies with different sera. Thus one serum may have an index of 3, in which case .03 c.c. of serum is used in each tube in making the test. Another serum, having a hemolytic index of 15, will have .15 c.c. of serum added to each tube. We anticipated that this would be a serious objection and that this larger amount of serum, when used in our routine Wassermann test, would give different results than would be obtained in using the ordinary amount, i. e., .02 c.c. Accordingly, series of Wassermann tests were made in which the indexed amount of serum was employed after being inactivated, this series being controlled by the routine Wassermann test, using .02 c.c. of serum. In no case in which the latter gave negative results did it change to positive when the larger amount of serum was used, even though the modified test gave a posi-

tive reaction. In a few positive cases, a slightly stronger positive was obtained, for example, one giving + + + with .02 c.c. of the serum might give + + + + when the indexed quantity was employed. Our results indicate that no material change would have been obtained by the routine Wassermann method if the indexed amount of serum had been used in each case. The rapidity of fixation of the patient's complement in the presence of lipoidal antigen and syphilitic antibody led us to try a similar technic, using the complement of guinea pig serum and antishoop amboceptor of serum from immune rabbits, with no preliminary period of incubation for complement fixation. Both the amboceptor and complement were carefully titrated and two series of tests were set up. In one of these, one unit of complement and one unit of amboceptor were added to each tube. In the second series, two units of each were added to each tube. The inactivated serum to be tested, antigens and sheep corpuscles were then added in the same quantities as in the routine Wassermann test, and placed in the water-bath at 37° C. for thirty minutes. In the first series, in which one unit only of complement and one unit of amboceptor were used, the serum controls hemolyzed while all of the tubes containing cholesterinized antigen, whether negative or positive by the routine Wassermann test, failed to show hemolysis, and several of the negative sera, tested by the other antigens, showed only partial hemolysis. Using two units each of complement and of amboceptor, all of the serum controls hemolyzed and most of the tubes containing negative sera, in which the antigen was present, also hemolyzed. A few failed to hemolyze. The tubes containing the positive sera, which also contained antigen, did not hemolyze. There had evidently here been a rapid complement fixation as in the modified test described, but it was much less clear cut, and an excess of complement and amboceptor was necessary in order to produce hemolysis. The results are evidently much more delicate and reliable when natural complement and the antishoop amboceptor of the patient's own blood are used than they are when the guinea pig complement and rabbit antishoop amboceptor are employed.

#### SUMMARY.

A modification of the Wassermann test is described which employs the natural complement and the antishoop amboceptor of the patient's own serum and in which there is no preliminary incubation at 37° C. for complement fixation. The method has been found to be more delicate than the routine Wassermann test and, according to the results so far obtained, is more reliable. It is not advocated as a method to replace the routine Wassermann test, but one to be given a trial paralleled with that test until its value is determined.

# MEASUREMENT OF THE SPINAL PUNCTURE NEEDLE

BY A. LEVINSON, M.D., CHICAGO, ILL.

IN the course of recent research on spinal fluid it was thought worth while to measure the part of the needle introduced into the spinal canal for spinal puncture. Such a procedure we felt would enable us to ascertain whether the length of the needle was constant at different ages, and whether it was possible to standardize the length—information that would be of value to the one performing the puncture. We interested ourselves particularly in the measurement of the needle in spinal punctures performed on children.

In consulting literature on the subject very little mention of the length of the needle was found, the only tabulated information being that of Quincke, the

TABLE I  
MEASUREMENTS BY QUINCKE

AGE	DEPTH
11½ years	2. cm.
7 "	2.5 "
	3. "
	3. "
	2.7 (3.5) cm.
	3.2 "
2 "	2.5 "
1¼ "	2. "
25 "	1.5 "
	6. "
	6.5 (4.7) "
39 "	5 plus "
22 "	5.2 "

originator of spinal puncture (Table I). Quincke, however, cites only a few cases.

In measuring the spinal puncture needle we proceeded as follows:

After obtaining a good flow of fluid the needle was removed from the spinal canal. It was grasped between the thumb and the index finger close to the patient's skin, and pulled out. The needle was then measured from the point held by the fingers to the tip, expressing the measurements in centimeters.

In Table II are tabulated the measurements of the needle made on children ranging from four months to twelve years. In Table III are tabulated the measurements of the needle made in adults of various ages. Owing to lack of space we are including but a few of several hundred measurements made. In cases where the results at the same age were similar, but one measurement is tabulated; in cases where they were different, several of a given age are tabulated. In some cases the results of several measurements are given for the same patient to show the length of the needle at different punctures.

As is seen from the above tables, the spinal puncture needle shows a length varying from 2.0 to 4.0 cm. in children up to the age of twelve, and a length varying from 4.1 to 10.0 cm. in patients over sixteen years of age. The large variations shown in the adult chart may be attributed to several factors.

TABLE II

MEASUREMENTS OF NEEDLE IN CHILDREN			
NAME	AGE	LENGTH OF NEEDLE IN CM.	
R. R.	4 months	2.0	
R. D.	5 "	2.2	
A. J.	6 "	2.0	
A. S.	6 "	2.3	
I. G.	6 "	2.5	
E. H.	7 "	2.5	
E. G.	8 "	2.4	
E. G.	9 "	2.2	
A. C.	12 "	2.2	
S. K.	12 "	2.0	
A. N.	14 "	2.8	
A. N.	14 "	2.5	
J. S.	21 "	3.5	
M. M.	22 "	3.0	
T. K.	2 years	2.7	
T. K.	2 "	2.1	
R. P.	2 " 7 mos.	2.4	
F. W.	3 "	4.0	
J. M.	3 "	2.0	
M. H.	3 "	2.8	
M. K.	3 "	2.9	
McM.	4 "	3.2	
H.	4 "	2.9	
P. O.	4 "	2.8	
W. J.	5 "	3.2	
R. L.	6 "	3.0	
A. G.	7 "	4.0	
G. M.	8 "	3.5	
S. L.	8 "	3.5	
S. B.	9 "	3.7	
S. B.	9 "	3.4	
S. B.	9 "	4.0	
E. D.	9 "	3.8	
R. A.	10 "	3.6	
L. G.	12 "	3.2	
L. G.	12 "	3.6	

Physique, thinness or fat, is one factor. Another factor in determining variations in the length of the needle is the direction taken by the operator in inserting the needle; an oblique insertion naturally requiring a greater length of needle than a straight insertion. The location of the interspace also determines the length of the needle, some clinicians using the space between the 2nd and 3rd vertebræ as the place of introduction, and others the space between the 1st and 2nd vertebræ. The higher the point of insertion the greater the measurement of the needle. Of interest is the fact that the length of the needle varies at different punctures in the same patient. This is due to the fact that the canal is quite wide and that fluid may be removed whether the needle is near the anterior or near the posterior surface of the canal.

## CONCLUSION

Although it is difficult to establish an exact standard as to the length of the needle in lumbar punctures, it is possible to gain an approximate estimate as to the measurement of the needle in patients of different ages. Our investigation showed a length varying from 2 to 2.5 cm. in children under one year of age;



TABLE III

MEASUREMENTS OF NEEDLE IN ADULTS		
NAME	AGE	LENGTH OF NEEDLE IN CM.
S. G.	16 years	5.4
T. R.	20 "	4.8
C.	25 "	5.0
P.	26 "	5.3
K.	27 "	5.8
Mrs. D.	29 "	4.9
L.	29 "	5.5
A. C.	30 "	5.6
E.	30 "	4.5
D.	30 "	5.4
H.	30 "	5.2
E.	30 "	5.8
A. C.	30 "	5.6
T.	31 "	5.5
J.T.	31 "	10.0 (very fat)
Mrs. D.	32 "	5.1
A. C.	33 "	4.1
L. N.	33 "	5.1
M.	34 "	5.1
P.	34 "	5.2
S. G.	34 "	4.5
S.	34 "	4.6
M.	35 "	5.7
M.	35 "	4.8
R.	35 "	5.5
H.	36 "	4.1
M. W.	37 "	5.3
I. G.	37 "	5.5
D.	37 "	5.9
S. S.	38 "	5.0
J. J.	39 "	7.0 (very fat)
S.	40 "	6.0
P.	40 "	6.4
M.	40 "	4.9
W.	40 "	5.0
R.	40 "	6.4
E.	42 "	5.0
W.	43 "	6.3
K.	43 "	4.5
M. B.	44 "	4.9
J. A.	44 "	5.8
W.	44 "	5.5
S.	45 "	5.5
F.	46 "	4.9
Mrs. B.	48 "	4.9
M.	48 "	5.3
L.	48 "	5.5
S.	52 "	5.2
R.	53 "	5.1
B.	53 "	10.0 (very fat)
B.	53 "	6.0
H.	54 "	5.2
W.	55 "	5.3
S.	55 "	6.7
D.	57 "	5.4
A.	57 "	5.6
M. R.	60 "	4.1
F.	60 "	5.9
K.	60 "	6.0
B.	60 "	5.9
S.	61 "	5.9
W.	65 "	8.0

2.0 to 3.5 cm. in children between one and two years of age; 2.0 to 4.0 cm. in children two to four years old; 2.8 to 4.0 in children four to seven years old, and 3.2 to 4.0 in children 7 to 12 years old. After the age of sixteen, the variation ranged from 4.1 to 10 cm., the greatest number running between 4.5 and 5.8 cm.

## A DEVICE FOR ACCURATE PIPETTING\*

BY LEONARD R. THOMPSON, B. S., NAPA, CALIF.

THE apparatus consists of a large syringe, two stands, one burette clamp, one spring clamp of a special kind, a clamp attachment to fasten the spring clamp to a stand, and various types of pipettes.

The syringe should be one of 20 or 25 c.c. capacity, and preferably, although not necessarily, one with a heavy, solid glass piston. The piston must fit the barrel of the syringe well. The piston, just previous to use, is covered with a thin coat of vaseline to insure smoothness of movement, and to keep it stationary when so desired. On the nipple, ordinarily used for attaching a needle, is placed a short piece of rubber tubing of good quality, and about one and one-half inches in length.

The syringe is fixed, by means of the burette clamp, to one stand in horizontal position. This stand, unless it has a heavy base, should be weighted to steady the apparatus. The mouth end of the pipette is inserted securely into the rubber tubing. The end of the pipette is best when tapered, as shown in the accompanying illustration, to facilitate slipping it into the rubber tube. The pipette is clamped securely to the other stand in a vertical position by means of the spring clamp. This clamp may be easily made of two pieces of wood, and a strong rubber band. The jaws are toothed to hold the pipette more firmly, and they are beveled at the end to make it easier to slip the pipette in. The diagram explains its simple construction. The rubber connection between pipette and syringe must be perfect, otherwise, the pipette will not hold the fluid. The pipettes used may be of a variety of types, varying from small capillary pipettes to large graduated pipettes.

The apparatus is operated in the following manner: The piston is pushed in to within a short distance of its entire length, but not its entire length. The fluid to be measured is brought to the pipette tip, and the plunger of the syringe drawn out until the desired amount of fluid is in the pipette. The fluid is then delivered in the required amounts into the receptacles placed under the pipette, by revolving the piston on its long axis and at the same time gently pushing inward. With this arrangement perfect control of the quantity to be delivered is secured.

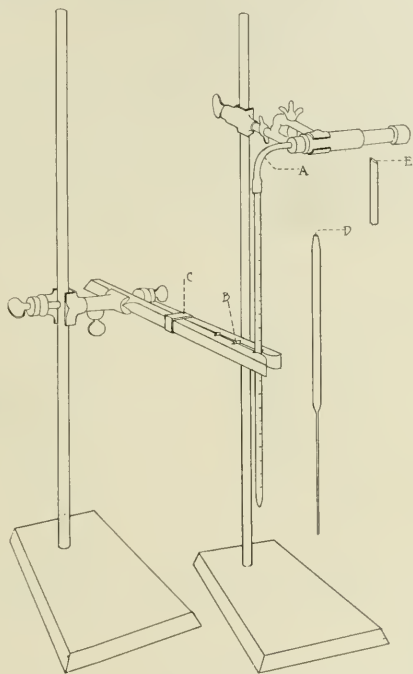
The writer finds the apparatus to be very useful in performing the Wassermann test, the Widal test, and other work in which accurate measurements of

---

\*From the Napa State Hospital, Napa, Calif.

small and large quantities are necessary. In the Wassermann test, one-tenth cubic centimeter quantities of serum are delivered with the greatest ease and accuracy. If the pipettes are tapered at the mouth end, they may be changed with great rapidity so that there is no time lost in this process. The device has the advantage of delivering with ease the reagents directly into the bottom of the tube. It is easily controlled, because the pipette is always in a position where a perfect view of it may be had. This is important in withdrawing serum from small quantities of blood.

The writer uses a graduated ten cubic centimeter pipette for delivering complement, salt solution, cells, and amboceptor. By this means these reagents



A device for accurate pipetting. *A*, rubber tubing; *B*, toothed jaw of wooden spring clamp; *C*, stout rubber band; *D*, capillary pipette with sloping end for insertion in rubber tube, *A*; *E*, beveled end of pipette for easy insertion in tube, *A*.

are delivered in the desired amounts accurately and rapidly. The following illustrates the usefulness of the apparatus in Widal tests. Recently the writer desired to deliver one capillary drop of serum to each of twelve tubes from a quantity of fourteen such drops. The twelve drops were delivered with two drops remaining in the capillary pipette.

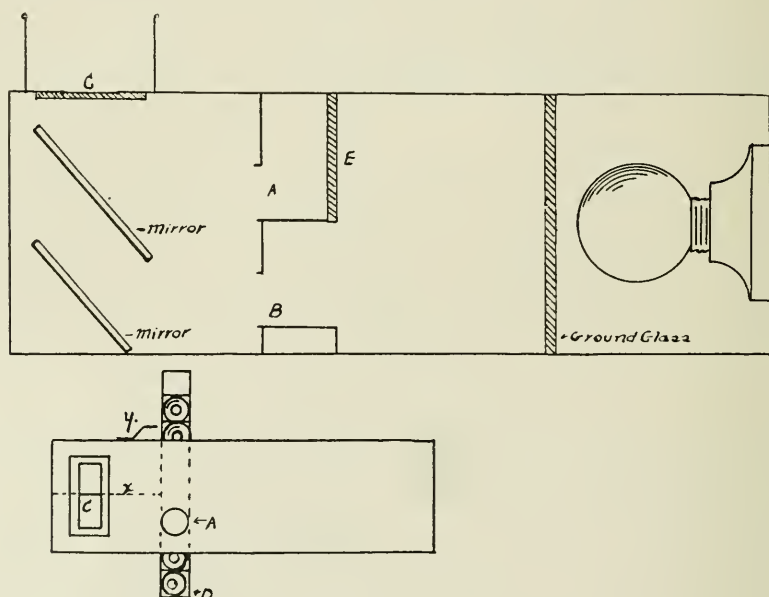
It is hardly necessary to point out that the use of this apparatus removes the disagreeable task of pipetting syphilitic serum, typhoid cultures, chemicals, and the like, by means of the mouth method.

## AN INEXPENSIVE COLORIMETER\*

BY J. W. WEIR, OKLAHOMA CITY, OKLA.

THE colorimeter described was constructed because of the demand in our laboratory for an instrument that would be capable of giving accurate figures and yet would allow of more rapid manipulation than the types now being sold. It can be constructed by any person who can handle tin shears and a soldering-iron, while its accuracy is essentially a question of the accuracy with which the standards are constructed.

The instrument consists of a metal box, containing an illuminating globe at one end in front of which is placed a sheet of ground glass to diffuse the light. The samples are placed in front of opening *A* through a hole provided in the top of the box. A rack, containing standard bottles, slides through openings provided in the sides of the box, and passes before opening *B*.



The light rays passing through bottles in front of *A* and *B* are reflected from mirror surfaces onto ground glass *C*. These mirrors are separated by a metal partition *X* which also serves to divide *C* into two surfaces. A metal extension surrounds the ground glass *C* in order to eliminate external light rays. Colors produced in a sample can be matched very accurately by the standards, since the two illuminated fields are so close together. Also, the eye is not confused by a multiplicity of standards being in the range of vision at one time.

A pointer *Y* is soldered to the side of the box and indicates numerals on the side of the standard rack which express the value of the standard bottle in

\*From the Clinical and Research Laboratories of the Wesley Hospital, Oklahoma City, Okla.



front of the opening *B*. This allows a quick determination of just which standard is in position within the box. It will be found necessary to place an extra sheet of ground glass in front of sample at opening *A* since the rays passing through this opening traverse less space in reaching the ground glass *C*. This additional piece of ground glass should be adjusted to or from the source of illumination until both halves of ground glass *C* are evenly illuminated.

We are using this instrument in the determination of blood and urine sugar, creatinine, alkali reserve; also in the preparation of standard strength vaccines; in fact, for any work in which the standards are transparent enough to pass light rays. The standards in each case were prepared from known solutions of the material to be determined, and were then matched by inorganic coloring materials in order that a permanent standard be obtained. For instance, potassium bichromate solutions are used to simulate the color reactions produced by creatinine. The standard racks are constructed to carry the number of standard bottles which will cover the possible range which is to be found in the materials tested. The instrument, as constructed by us, uses two cubic centimeters, vaccine ampules, these giving approximately one-half inch of fluid for comparison.

# *The Journal of Laboratory and Clinical Medicine*

VOL. III.

NOVEMBER, 1917

No. 2

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.  
Ann Arbor, Mich.

## ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D.	- - -	ST. LOUIS
HANS ZINSSER, M.D.	- - -	NEW YORK
PAUL G. WOOLLEY, M.D.	- - -	CINCINNATI
FREDERICK P. GAY, M.D.	- - -	BERKELEY, CAL.
J. J. R. MACLEOD, M.B.	- - -	CLEVELAND
ROY G. PEARCE, M.D.	- - -	CLEVELAND
ROGER S. MORRIS, M.D.	- - -	CINCINNATI
GERALD B. WEBB, M.D.	- - -	COLORADO SPRINGS
E. E. SOUTHARD, M.D.	- - -	BOSTON

Contents of this Journal Copyright, 1917, by The C. V. Mosby Company—All Rights Reserved  
Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

## EDITORIALS

### *Disordered Action of the Heart Among Soldiers*

THE British Medical Research Committee has issued a report upon this subject prepared by Lewis, the expert on the heart. The urgent need for the services of every fit man in the war has apparently led to a close study of disturbances of the heart's action as disqualifying men for military duty. To tell anyone, and especially a soldier, that he has heart disease or even a functional disorder of this organ, is likely to disturb his equilibrium and impair his effectiveness. British army officers have been in the habit of labeling their cases D.A.H. (disordered action of the heart) or V.D.H. (valvular disease of the heart), but these symbols do not deceive Tommy Atkins, and often mislead his physicians because they do not, in a large proportion of cases, give a correct idea of the true condition. It will be remembered that as long ago as our Civil War, medical officers used the indefinite designation of "irritable heart," which has been continued more or less in civil life.

British medical officers now propose the term "effort syndrome," which is found in a variety of pathologic states, as in early heart disease, in early tuberculosis, and in exophthalmic goiter. But it is also found with much greater frequency in soldiers without signs of structural change in any organ. Refer-

ence to the heart prejudices not only the treatment of the soldier, but to a large degree, his return to duty.

The "effort syndrome" comprises the following symptoms, stated in the order of their importance: (1) Breathlessness, exaggerated by exercise or emotion, is a constant symptom. Occasionally it is manifest at rest, rarely in paroxysms. (2) Pain is not constant, but frequent, varying in degree from precordial discomfort to anginal in character and distribution; especially intensified by exercise. (3) Exhaustion, usually provoked by sustained effort, is an almost constant symptom. It is in excess of that due to fatigue in healthy men. It may be a continuous symptom, and it is not only physical, but mental. (4) Giddiness is almost constant and is associated with change of position and increased effort. Fainting is less common and owes its chief importance to its incapacitating effect. (5) Palpitation is frequent, especially during and after increased effort. (6) Headache is frequent, of the frontal, throbbing type, and often severe after increased effort. (7) Lassitude, even at best, is often present, while coldness of hands and feet and swelling of the extremities and excessive perspiration of the body are common. (8) Irritability of temper, sleeplessness, shakiness, and flushing are common, especially the three last named. A disinclination to take alcohol in any form, sometimes for conscientious reasons, but as commonly from distaste, is a frequent and remarkable association.

The signs found on examination are equally distinctive and are as follows: (1) Increased heart rate is observed under all circumstances, but the characteristic fact is that the increase under emotion, exercise, and posture is greater than in normal subjects. (2) The systolic pressure is unduly increased by exercise or emotion. (3) Diffusion of the apex beat to several rib spaces and forcible or jerky impulses are frequent, as is also concentration of the heart sounds. (4) Intermittence of the heart or irregularity accompanying respiration, especially when deepened, is not uncommon. (5) Temperature charts for the most part are normal, but slight and fleeting elevations as high as  $99.5^{\circ}$  or even  $100^{\circ}$  are not rare. (6) Exercise causes exaggerated respiration. (7) The palms and soles are frequently wet and cold, while tremor of the hands is the rule. (8) The urine is strongly acid and frequently deposits oxalates on cooling. (9) The capillary leucocytic count is high.

Cardiovascular experts sort out their cases as follows: In a preliminary examination the following groups are eliminated as unfit for service: (a) Those in whom signs of pulmonary tuberculosis or frank exophthalmic goiter are discovered. (b) Those who present unmistakable signs of heart disease; namely, instances of well-defined initial stenosis, aortic regurgitation or aneurysm; instances in which there is an appreciable displacement of the heart's apex beat to the left; instances in which there is a grave form of cardiac irregularity.

In regard to cardiac murmurs, the following statements are made: "When a soldier presents the characteristic low pitched rumbling murmur in diastole and at the apex beats, or when an early diastolic murmur, maximal at the level of the second costal cartilage, is associated with the water-hammer pulse, then, by common consent, he is unfit for duty. Instances of systolic murmurs at base of apex can not be treated similarly. In the absence of other disqualifying signs

or symptoms it is wise entirely to neglect such murmurs in soldiers. This conclusion is at variance with much current teaching, and the reasons for insistence upon are, therefore, given in full: (a) Systolic murmurs at base of apex indicate valvular lesions only exceptionally; there is no conformity of opinion as to the character or conduction of systolic murmurs indicating valvular lesion. (b) The extent of mitral valve damage which produces a systolic murmur alone is relatively slight; the disease is often limited to the valve, the heart's muscle which is the essential part of the organ being wholly undamaged. (c) Patients who are invalided on the ground of systolic murmurs *alone* are subsequently found *when tested* to be fit for active service in nearly all instances. A large number of men who present such murmurs are known to have passed the most severe ordeals of active service without accident. (d) If a group of patients who present no murmurs and a similar group in whom systolic murmurs exist are tested in regard to efficiency in work, no difference is to be found in the capacity of the two groups. The estimate of fitness or unfitness for service can be gauged with considerable accuracy without reference to such murmurs; as soon as murmurs of systolic time are taken into consideration the issue becomes confused.

*Auscultation is the least valuable method employed in sorting soldiers suffering from cardiovascular derangements; the sorting can almost always be effected without its aid.*

With reference to cardiac enlargements, the following is given: "An appreciable increase of dullness to the left of the sternum, a strong impulse beyond the nipple line, are the most satisfactory guides to enlargement. An extension of the impulse to several rib spaces is emphatically unreliable as a sign of cardiac dilation (as tested by accurate x-ray measurements). In estimating the size of the heart, allowance for the weight of the man should be made. Enlargement, when discovered, is a clear indication of future incapacity." Concerning cardiac irregularities the following is said: "Intermittence of the pulse and regular groupings of the pulse beats should not be taken into account; they are not physical signs of heart disease and do not incapacitate. The only irregularity of consequence in soldiers is a persistent irregularity of a very disorderly type, *which does not disappear when the heart is accelerated during or immediately after exercise*. As to the detection of early heart disease, the following statement is made: "The fear of overlooking early heart disease, and the widely felt difficulty of its diagnosis is chiefly responsible for hesitancy in dealing with the men, and for blunders in classing them for duty and discharge. Here again actual experience is the only safe guide. It may be taken as an axiom that no soldier, who is free from symptoms on duty, has an affection of the heart which incapacitates him, and this axiom may be adopted irrespective of any unusual sign found in the heart. Now, actual heart diseases in young soldiers, in the absence of a history of rheumatic fever or syphilis is a comparative rarity. When there is a history of rheumatic fever, and especially when symptoms date from rheumatic fever, the question of diagnosing a heart lesion need only rarely arise; for, whether it exists or not, the history and symptoms alone will almost always declare incapacity; it is a matter of experience that these men rarely re-



turn to active service, and if they return, the subsequent stay in hospital will greatly outweigh the duration of duty performed. In regard to syphilis, it is reasonable that among those who exhibit signs and symptoms of the syndrome considered, a history of syphilis or a positive complement-fixation test is very rare. Venereal diseases, like alcoholism, is conspicuous by its absence from the histories of these soldiers. When the first sorting has been accomplished upon the lines indicated, any further attempt to diagnose organic disease of valves or muscles should not be attempted, for, if attempted, it will in the best of hands prove unprofitable; in inexperienced hands it will often prove disastrous."

A second, rapid sorting leads to the elimination of the following groups: (1) Those in whom the onset of symptoms dates from rheumatic fever, or in whom there has been recurrent rheumatic fever. (2) Those in whom breathlessness on exertion is found to be persistently severe. (3) Those in whom precordial pain prevents exercise. (4) Those with a persistently high pulse rate (120 or above), even when recumbent. (5) Those in whom a single exercise test, such as ascending 30 steps, produces objective signs of distress, an anxious expression, a respiratory rate of 35 or over which persists while the patient lies, or a pulse rate which fails to fall within five beats of the pre-exercise level in lying for two minutes. (6) Those in whom the symptoms are moderate, but have been of many years' duration. This class includes those who pass up games in school or who left heavy work in civil life on account of symptoms.

A final sorting follows subjecting the men to graduated exercise and observing the effect. "That a man's observed capacity to accomplish work of a given order is the only dependable test of such capacity would seem self-evident; yet it is the experience that medical officers rely more upon physical signs obtained while the subject is at rest. A final sorting can not be accomplished efficiently in this manner. In selecting a candidate for a post as typist, it is not only by questionings, it is not by examining the configuration of the hands or the electrical response of the muscles that the desired knowledge of deftness and accuracy in working is to be obtained. The decisive test is an exercise upon the machine which will be used. It is true that specific anatomic defects may divulge incapacity; but it is equally true, on the one hand, that anatomic imperfections do not necessarily unfit; and, on the other hand, that seeming anatomic perfection is no criterion of manipulation, power, or skill. The heart provides no exception from these clearly sound propositions."

—V. C. V.

### *Tuberculosis and the Army*

IT is stated that since 1865 the death rate from tuberculosis has fallen with almost ever increased velocity. Cobbett<sup>1</sup> states that irregularities have occurred owing to fluctuations in the conditions of life favorable or unfavorable to consumptive persons. In favorable periods many consumptives may span their lives a few years further, whereas in unfavorable times these lives will be shortened. An extreme incident of this is quoted by Cobbett as occurring dur-

ing the siege of Paris in 1870-1871. There was an enormous mortality from tuberculosis during this period. A temporary fall from 1872 to 1880 followed this rise. The death rate from tuberculosis in Paris only in 1880 again reached the height it had averaged before the siege (1865-1880).

Such history it would appear is being repeated on a colossal scale in France during the present conflict. Biggs,<sup>2</sup> in an excellent account of his French investigations for the Rockefeller Foundation, has described the acceleration in the consumptive death rate due to the severity of modern warfare on troops none too carefully selected at its onset. France has always neglected her tuberculosis problems, and her death rate before the war from this disease was three times as great as that of England, which has the lowest tuberculosis rate of any great country. In Paris, as an example, one in every four deaths was certified as due to tuberculosis.

The study of data from the siege of Paris and data collected by Biggs further strengthens the conception, so well expounded by Bushnell,<sup>3</sup> Baldwin,<sup>4</sup> Fishberg<sup>5</sup> and others in this country, that pulmonary tuberculosis originates from bacilli already planted in the individual, rather than from tubercle bacilli from without or from another consumptive. As Krause<sup>6</sup> has so well expressed it, an individual is as safe against tuberculosis as the strength of the "capsule" or "shell" of the tubercle already planted in his body.

Stress, strain, exhaustion, and various infections can so weaken this defensive capsule that large or small numbers of tubercle bacilli may become distributed in a hypersensitized system and can then overwhelm or sicken the victim.

Probably as a result of the experience of France our Surgeon-General has wisely distributed Tuberculosis Boards composed of experts, to examine all men in the United States Army, National Guards, and Drafts. Such boards are, we understand, finding that about one per cent of the soldiers have somewhat advanced pulmonary tuberculosis, and about four per cent have quiescent pulmonary lesions. If five thousand such men are disqualified, out of an army of half a million, great good will be done for these men and also for the government. The four per cent of men with inactive lesions will probably continue in service, and time will tell of their future.

It is very doubtful if medical science can yet boast of great refinement in the art of diagnosis of pulmonary tuberculosis. In other words, no method is yet available for estimating the strength of the "capsule" in the tuberculizable. Physique is a poor criterion, for we all know how athletes and robust looking men can quickly succumb.

Soldiers with advanced lesions, who are now being weeded out, can often be detected with little physical examination; at times, merely by inspection. There will undoubtedly be more than the four per cent, marked with slight lesions, who will suffer from tuberculosis in the next few years, and many so listed will not succumb. In peace times with a standing army of about seventy-five thousand men, Fort Bayard is usually full with a quota of four hundred odd consumptives. What will be the result on our troops from the stress of trench warfare can not yet be foretold. England brought her army together

slowly and carefully, and it is understood that so far no serious numbers of tuberculous men are being cared for. Canada has had about one per cent of her men returned with active disease. The outbreak of pulmonary tuberculosis, which must occur in the United States Army, will be watched with more than usual interest during the next few years.

## BIBLIOGRAPHY

<sup>1</sup>Cobbett: The Causes of Tuberculosis, Cambridge, 1917.

<sup>2</sup>Biggs: The War Tuberculosis Problem for the Nation. Am. Review Tuberc., July, 1917.

<sup>3</sup>Bushnell: Mil. Surgeon, 1913.

<sup>4</sup>Baldwin: The Consumptive and His Neighbors, The Survey, 1917.

<sup>5</sup>Fishberg: Pulmonary Tuberculosis, 1916.

<sup>6</sup>Krause: The Nature of Resistance to Tuberculosis, Am. Review Tuberc., April, 1917.

—G. B. II.

### *Lymphocytes and Cancer*

IN recent editorials we have reviewed the theory and experiments of Murphy<sup>1</sup> and his collaborators on the function of the lymphocyte in cancer and tuberculosis. Murphy, it will be remembered, considers the lymphocyte an important factor in the resistance of the individual to both these diseases, and his experimental work, and that of his colleagues has seemed quite convincing. Nevertheless, the recent reports of Sittenfield and Stevenson cast some doubt upon the valuable activities of these cells of lymphoid type.

Sittenfield<sup>2</sup> concludes, from his experiments, that a high degree of lymphocytosis, caused either by subcutaneous injections of pilocarpin or by intravenous introduction of leucocytic cream from rats that had received stimulating doses of x-ray, affords neither a protective nor a defensive mechanism against tumor inoculation. He also says that naturally immune rats, subjected to repeated doses of x-ray which caused a very low lymphoid cell content of the blood, remained refractory to the Flexner-Jobling tumor; and further, he states, neither increase nor decrease of the lymphoid elements in the blood had any influence upon either resistance or susceptibility to tumor growth.

It had been noticed in the earlier experiments of Murphy that tumor cells grew well in embryos during the period before the appearance of cells of the lymphoid type. It was this observation which led to the later series of experiments. But now comes Stevenson,<sup>3</sup> who, after a series of experiments, concludes that all the tumors he used grew without hindrance in the chick embryo in the presence of adult chicken spleen.

As is too often the case, there now exist two series of researches, dealing with the same problem, and diametrically opposed to one another. It may be that both are true. It may be that the methods of handling the tissues, or the time relations in the two series of experiments are essential in giving such different results. It is possible that the lymphocytic reaction in cancers is totally extraneous, so far as the resistance reactions of the hosts are concerned, and that the basic fact is one which concerns the physicochemical state of the inoculated material. The work of Novy<sup>4</sup> opens new fields when one is dealing with

colloids. In this connection one might recall the fact that sterile filtrates of Rous' chicken sarcoma cause tumors, and that this phenomenon leads Ewing<sup>5</sup> to believe that in that instance one is dealing with a chemically transmissible virus.

## BIBLIOGRAPHY.

<sup>1</sup>Jour. Lab. and Clin. Med., 1916, i, 538; Ibid., 1917, ii.

<sup>2</sup>Sittenfield: Jour. Cancer Research, 1917, ii, 151.

<sup>3</sup>Stevenson: Jour. Cancer Research, 1917, ii, 245.

<sup>4</sup>Novy: Jour. Infect. Dis., 1917, xx, 536, 566, 589, 618, 629.

<sup>5</sup>Ewing: Jour. Cancer Research, 1916, i, 71; Jour. Lab. and Clin. Med., 1916, i, 625.

—P. G. W.



# *The Journal of Laboratory and Clinical Medicine*

VOL. III.

ST. LOUIS, DECEMBER, 1917

No. 3.

## *ORIGINAL ARTICLES*

### STUDIES ON CHOLESTEROL

#### IV. EXPERIMENTS CONCERNING THE RELATION OF THE DIET, THE BLOOD CHOLESTEROL, AND THE "LYMPHOID DEFENSE"

BY GEORGINE LUDEN, M.D., ROCHESTER, MINN.

IN a series of experiments on myself made between June 17 and Dec. 30, 1916, I was able to observe a close relation as well as a reciprocal action between the diet, the blood cholesterol, and the lymphocytic reaction or "lymphoid defense." The term "lymphoid defense" was chosen in reference to the observations of Murphy and Morton, who found that animals on which cancer transplants did not "take" showed up to a 200 per cent increase in the lymphocyte count of their blood, while those not endowed with this natural resistance showed no such increase. When the lymphoid tissues were injured or partly destroyed by means of roentgen rays 100 per cent of "takes" could be obtained. The term "lymphoid defense" will be used in this paper to represent the combined percentages of the small and the large lymphocytes in the differential counts. The transitionals have not been included, as hematologists do not agree concerning the place that should be assigned to them in the classification of the blood cells, though they may play a part in the reaction against malignant proliferation. Grawitz considers transitionals to be the prototypes of the polymorphonuclear neutrophil leucocytes, Simon classifies them with the large lymphocytes, and Ziegler<sup>1</sup> looks upon them as closely allied to lymphoid myelocytoid cells.

The relation observed between the diet, the blood cholesterol, and the lymphoid defense suggested that beneficial results might be obtained from dietary measures different from those we are accustomed to take into account. The practical importance of this relation may be gathered from the following considerations:

\*From the Mayo Clinic, Rochester, Minn.

Murphy and Morton state that the "lymphoid crisis" which they describe is to be considered "not merely an accompanying factor in the immune period, but essential to the process." In other words, the lymphocytic reaction appears to be one of the body's natural means of defense against malignant proliferation. This assumption seems to be corroborated by Stevens' recent observations on a reactive lymphocytosis induced by roentgen rays and followed by improvement in cases of malignant growth. The fact that "lymph gland extract" is being tried against malignant tumors and conditions marked by new growth of tissue, also tends to show that Morton and Murphy's interpretation of the lymphocytic reaction is gaining acceptance in medical circles.

The physiologic activity of cholesterol and the cholesterol content of the blood have been the object of a great number of recent investigations as regards their relation both to normal growth and to various pathologic conditions, by Robertson,<sup>40</sup> Browder, Luden,<sup>28</sup> Robertson and Burnett,<sup>39</sup> Rothschild,<sup>42, 43, 44, 45</sup> Bloor,<sup>3, 4, 5, 6</sup> and others. Luden<sup>28</sup> has called especial attention to the effect of cholesterol retention on cell proliferation.

The influence of the diet on the cholesterol content of the blood, which had been studied previously in animals because of its relation to arteriosclerosis by McMeans, Saltykow and others, or its relation to the function of diverse organs (the adrenals, Krylow, Sternberg; the spleen, Soper; the liver, Chalatow, Rothschild, and others) has lately been given a clinical application by Rothschild in his work on the dietetic management of hypercholesterinemia in cases of cholelithiasis. This work shows that dietary measures calculated to reduce the blood cholesterol will relieve the symptoms of a group of patients to whom cholecystectomy, and often secondary operations have brought only temporary relief.

So far as the writer is aware, the relation between the diet, the lymphoid defense, and the cholesterol content of the blood has never been studied, yet the deduction seems admissible that dietary measures calculated to reduce the blood cholesterol while stimulating the lymphocytic reaction might prove as beneficial in cases of carcinoma before and after operation as the regulation of the diet in cases of chronic hypercholesteremia after cholecystectomy. The good results obtained by postoperative treatment of malignant growths with radium and roentgen rays are well known, and Stevens' observations suggest that these results may be due to the lymphocytic reaction induced by the rays. In a small number of cases which the writer has had the opportunity to study, both radium and the roentgen rays appeared to increase the lymphocyte count while they lowered the cholesterol content of the blood. The latter observation was reported also by Luden<sup>29</sup> in connection with the effect of experimental roentgen treatment on the blood of goats. There is no reason to suppose that the activation of the lymphoid defense by dietetic measures should be less beneficial than that produced by means of radium or the roentgen rays. On the contrary, it is to be expected that therapeutic measures that keep within the bounds of normal physiologic activity, so to speak, will be less apt to tax the general health of a patient than any extraneous measures.

Before describing the experiments with which this paper is concerned, it will

be necessary to discuss three factors that were bound to exert a great influence on the results obtained; namely, the individual cholesterol standard of the blood, the cholesterol content of certain articles of food, and the effect of digestion on the cholesterol content of the blood.

#### THE INDIVIDUAL CHOLESTEROL STANDARD OF THE BLOOD

Rothschild<sup>42</sup> has pointed out that normal animals have an individual cholesterol standard, the variations of which are slight. Luden<sup>29</sup> was able to corroborate

TABLE I

BLOOD CHOLESTEROL OF THE WRITER, NOV. 23, 1915, TO JUNE 19, 1916\*

DATE	AUTENRIETH (MG. CHOLESTEROL IN 5 C.C. CHLOROFORM)	BLOOR I (MG. CHOLESTEROL IN 6 C.C. CHLOROFORM)	BLOOR II (MG. CHOLESTEROL IN 6 C.C. CHLOROFORM)
Autenrieth-Hellige Colorimeter			
Nov. 23, 1915	0.180		
Jan. 31, 1916		0.216	0.260
Feb. 1		0.272	0.300
Mar. 1		0.272	
9		0.272	
18		0.272	
25**		0.330	
29		0.330	
Apr. 6		0.300	
9		0.272	
17			0.330
22		0.272	
June 16		0.272	
17		0.272	0.330
Duboscq Colorimeter			
17		0.275	0.333
18		0.272	0.333
19		0.272	0.330

\*The technic used for these tests is identical with that described in Luden's<sup>29</sup> studies on the cholesterol content of the blood of goats, with the exception only that the Duboscq colorimeter set at 10 mm. and a standard test 0.400 mg. was used instead of the Autenrieth-Hellige instrument. It should be remembered also that all colorimetric estimations are influenced by the individual "color vision" of the operator, even when all other factors are identical; hence my "normal" cholesterol values which were found in a series of healthy persons read somewhat higher than the values quoted by Bloor.<sup>17</sup> It should be added that in a large series of tests run parallel with the Duboscq and the Autenrieth colorimeters the difference between the readings of the two instruments kept constantly within the following range: Duboscq 0.222, Autenrieth 0.216; Duboscq 0.275, Autenrieth 0.272, the greatest difference being 0.008 mg. Consequently the cholesterol values obtained while the Autenrieth colorimeter was still in use, do not need any correction. Details concerning these parallel tests and of cholesterol values found in health and disease will be published shortly.

\*\*Slight attack of appendicitis.

these findings by observations on the cholesterol content of the blood of goats. Similar observations were made regarding the cholesterol value of normal persons partaking of an ordinary mixed diet. My own cholesterol standard was established by a series of determinations made between Nov. 23, 1915, and June 16, 1916 (Table I). It remained practically constant at 0.272 mg. in 6 c.c. of chloroform extract made according to Bloor's original method<sup>3</sup> for the determination of blood cholesterol. The only considerable exception occurred during a very slight attack of appendicitis, when the cholesterol went up to 0.330 mg. It returned to normal, however, within a fortnight.

## THE CHOLESTEROL CONTENT OF FOODS

Data concerning the cholesterol content of the usual articles of food are extremely scarce, and so far as can be ascertained, no such determinations have been made by the Bloor method. For the sake of a fair comparison with the changes observed in the cholesterol percentage of the blood during any given diet it seemed important to establish the cholesterol percentage of the components of these diets by the same method by which the blood was tested. Table II shows the values found in samples of the food used during the diet experiments. It should be remembered that a number of factors are bound to play an important part in the cholesterol value of foodstuffs. The breed, the age, and the mode of feeding animals will not be without influence on the cholesterol content of the food the animals supply. Even different parts of the meat of the same animal may have slightly different values. The time of calving materially affects the richness of the milk and its percentage of cholesterol, and in the case of cheese, for instance, the special brand and the ripening may not be without effect. Absolute data can, therefore, be obtained only by an extremely large number of deter-

TABLE II  
CHOLESTEROL OF FOODS

FOOD	WRITER'S DETERMINATIONS. DUBOSQ COLORIMETER.		OLDER METHODS <sup>15</sup>	
	<i>Bloor I</i>	<i>Bloor II</i> (mg. cholesterol in 6 c.c. chloroform)	(PER CENT)	
Yolk of egg, raw, 3 gm.....	2.660	2.660	2.15	1.75
Yolk of egg, hard boiled, 49% water.....	2.660	2.660		
Yolk of egg, desiccated.....	4.000	4.000		
Milk, raw, 3 c.c.....	0.080	0.080		
Cream, raw, 3 c.c.....	0.185	0.185		
Butter, 3 gm.....	0.472	0.880*	0.190	
Butterfat .....			0.200	0.220
Maize .....			0.100	
Mushrooms ( <i>agaricus campestris</i> ).....	0.444	0.307	0.520**	
Mushrooms ( <i>agaricus campestris</i> ) with sauce	0.210	0.181		
Beef, raw, 3 gm.....	0.160	0.160		
Beef, roast .....	0.190	0.190		
Beef, smoked, dried .....	0.181	0.250		
Chicken, roasted, breast.....	0.380	0.380		
Fish, black bass.....	0.250	0.250		
Oatmeal .....	0.000**	0.000***		

\*Bloor II tests appear to contain some kind of coloring material.

\*\**Boletus edulis*.

\*\*\*Autenrieth method also negative.

minations. The writer is fully aware of this, but has decided to publish the findings in the following tables for two reasons: First, the cholesterol content of the various articles of food used during the experiments must be closely associated with the results obtained; second, since present data on the subject are either vague, (Rothschild,<sup>45</sup> for instance, states that eggs, cream, milk, and cheese are rich in lipoids, but gives no definite values) or obtained by older methods, it may be of general interest to know the approximate cholesterol percentage of at



least a few of the common articles of food obtained by the Bloor method, allowance being made for slight variations in different samples of the same kind of food (Table II).

In selecting the sample of fish, a very dry piece from the center near the spine was used and tested with blotting paper to see that no trace of grease was present. The difference between the percentages for raw and roasted beef is explained by the loss of water that occurred in roasting. It is interesting to note that the different tests of the yolks of eggs correspond closely. The tests were made with a Duboscq colorimeter set at 10 mm. and the standard cholesterol solution containing 0.400 mg. per 6 c.c. of chloroform used in our blood cholesterol determinations.

#### INFLUENCE OF DIGESTION ON THE BLOOD CHOLESTEROL

Bloor<sup>3</sup> states that in dogs the process of digestion and the chemical constituents of the food do not influence the cholesterol content of the blood to any appreciable extent. The effect of digestion in ruminating herbivora has been discussed in my work on the blood of goats. As far as could be ascertained no data have yet been published on the changes occurring in the blood cholesterol of normal persons during the digestion of the average mixed diet.

Three experiments were made to study the effect of the digestive process on the writer's cholesterol standard during the usual mixed diet. In every instance the first test was made before breakfast, as Bloor<sup>6</sup> has found that the postabsorptive condition eight to sixteen hours after the last meal is "practically the only time when the blood is free from the influence of ingested or mobilized fat." According to Bloor and Rothschild, cholesterol and fat metabolism are closely associated. Whatever may be the exact time required for elimination of the other lipoids from the blood, the results obtained in these experiments seem to indicate that the blood cholesterol returns to its "standard value" in approximately four hours even when a considerable amount of food rich in cholesterol has been consumed. Consequently the time allowed—twelve hours—would be amply sufficient to exclude the effect of digestion from the first test. The subsequent tests were made at one hour's interval each, with the exception of the first test after the midday meal, which had to be taken after two hours on account of the absence of those who took the blood. For each test 3 c.c. of blood were taken from the cubital vein by means of a graduated syringe fitted with a hypodermic needle, according to Bloor's method,<sup>3, 4</sup> the method used in all my experiments. The blood was boiled up immediately after it had been taken. It was found necessary to let a week elapse between the two experiments as the repeated introduction of the hypodermic needle caused the cubital veins—the left and right were used alternately—to collapse, small thrombi being formed which obstructed the lumina. Attempts to use the veins on the back of the hand gave unsatisfactory results, the caliber of most of these being too small, or, when sufficiently large, it was impossible to fix the vein itself and it slid aside when the needle was introduced.

The results shown in Table III were obtained in these experiments:

As may be seen from the foregoing experiments, a light luncheon consisting of bread with butter and tea or coffee does not appear to produce any marked changes in the blood cholesterol, or at any rate these changes have been eliminated within an hour. The tests made after the more substantial meals seem to show that the cholesterol content of the blood returns to its "normal standard" in approximately four hours even when as much as three-fourths of a pound of raw beef and the yolk of an egg have been consumed at one meal. This fact should be borne in mind in considering the results obtained from an exclusively meat diet. (See Table V.)

EXPERIMENTS SHOWING THE EFFECT OF THE DIET ON THE CHOLESTEROL CONTENT  
AND THE CYTOLOGY OF THE BLOOD

The following experiments were suggested by the current belief that there is a causative relation between the increased consumption of meat and the incidence of cancer. Many writers do not hesitate to endorse this view. Williams writes: "I am persuaded that the ascertained facts justify the belief that there is a certain relation between the conditions of nutrition and the incidence of cancer growth." Bulkley has repeatedly called attention to the influence of the diet on the course of malignant disease. Von Müller and von Bauer<sup>33</sup> in their clinical lectures both emphasized the fact that the increase of cancer in Bavaria might be due, in part at least, to increased consumption of meat among the Bavarian peasantry. Hoffman comes to similar conclusions from his cancer statistics.

Cytologic changes in the blood in malignant disease have been reported by

TABLE III

HOURLY TESTS OF CHOLESTEROL IN WRITER'S BLOOD DURING THE PROCESS OF DIGESTION

FIRST EXPERIMENT, AUG. 2, 1916

Blood taken before breakfast showed a cholesterol value of 0.272 mg. by the Bloor I test and 0.333 mg. by the Bloor II test; that is, the usual interval between the two tests previously observed in the postabsorptive period.\*

Breakfast (8 A.M.)

2 egg sandwiches (1 egg)

$\frac{1}{2}$  cantaloupe

2 cups of tea (milk and sugar)

					DUBOSCQ COLORIMETER	
					Bloor I	Bloor II
					(mg. cholesterol in 6 c.c. chloroform)	
Blood taken	1 hour	after	breakfast	.....	0.347	0.420
"	"	2 hours	"	.....	0.333	0.380
"	"	3 "	"	.....	0.280	0.350
"	"	4 "	"	.....	0.272	0.330
Dinner (12 M.)						
1 cup cold broth						
1 "tartar" steak (raw)						
1 egg yolk						
1 small slice of bread with butter						
2 cups of coffee (cream and sugar)						
Blood taken	2 hours	after	dinner	.....	0.343	0.472
"	"	3 "	"	.....	0.363	0.500
"	"	4 "	"	.....	0.275	0.330

\*The cause of the interval between the two tests has been discussed elsewhere by the writer and can not be considered here.

TABLE III—CONTINUED  
SECOND EXPERIMENT, AUG. 7, 1916

Blood taken before breakfast showed 0.272 mg. cholesterol (Bloor I test) and 0.333 mg. (Bloor II test).

Breakfast (8 A.M.)

90 gm. raw beef  
1 small slice of bread with butter  
 $\frac{1}{2}$  cantaloupe  
2 cups of coffee (sugar and cream)

	DUBOSCQ COLORIMETER		AUTENRIETH-HELLIGE COLORIMETER	
	<i>Bloor I</i>	<i>Bloor II</i>	<i>Bloor I</i>	<i>Bloor II</i>
	(mg. cholesterol in 6 c.c. chloroform)			
Blood taken 1 hour after breakfast.....	0.330	0.338	0.358	0.342
" " 2 hours " " .....	0.285	0.363	0.284	0.362
" " 3 " " " .....	0.285	0.363	0.284	0.362
" " 4 " " " .....	0.272	0.333	0.272	0.330

Dinner (12 M.)

2 ham sandwiches  
1 cottage cheese sandwich  
1 egg sandwich ( $\frac{1}{2}$  egg)  
2 cups of coffee (sugar and cream)

Blood taken 2 hours after dinner.....	0.307	0.363
" " 3 " " " .....	0.272	0.333
" " 4 " " " .....	0.272	0.333

#### THIRD EXPERIMENT\*\*

Blood taken 4 hours after last meal showed 0.272 mg. cholesterol (Bloor I test) and 0.333 mg. (Bloor II test).

Light luncheon (4-5 P.M.)

3 small slices of bread with butter  
2 cups of tea (moderate amount  
of sugar and cream)

Blood taken 1 hour after luncheon.....	0.277	0.333
--	-------	-------

\*\*This experiment was made several times on different dates to determine whether patients could be allowed a light breakfast before their blood was tested.

Gruner, who based his work on the Arneith theory, and who succeeded moreover in reproducing these cytologic changes in his own blood by dietary measures.

Although some of Gruner's deductions may be open to criticism from a hematologic point of view, the main idea of his work and the honest effort which it embodied seemed to deserve recognition in a practical form; namely, the repetition of his experiment and the checking up of his results through independent investigation. Consequently certain of my experiments have been carried out along the lines suggested by him. Moreover it seemed possible that experiments of this kind might furnish data concerning the influence of the diet on the lymphocytic reaction and on the cholesterol content of the blood and, therefore, in a wider sense, on the relation of metabolism to the cancer problem.

Gruner has adopted a very detailed classification for the morphologic changes he observed in the leucocytes and lymphocytes of patients suffering from malignant conditions and in his own blood in dietetic experiments. Many of the pathologic nuclei he describes, however, somehow give the impression that they are perhaps artefacts due to accidents in staining or the mechanical effect of making the smear. Amitotic division, for instance, has been admitted for the lymphocytes and large mononuclears by Weidenreich; but some of Gruner's diagrams (for

example, Figs. 21 and 23) suggest a folding rather than an actual division of the nucleus. Two of his varieties of atypical neutrophil leucocytes, however, seem above suspicion and have, therefore, been taken into account in my experiments inasmuch as no mechanical effect could produce the peculiar shape of their nuclei. These are the "ringform" in which the nucleus is ring-like, and "bizarre forms" in which the nucleus represents a solid compact disc or bar which sometimes suggests the shape of the letter L or J.

Hematologists (Downey, Weidenreich) consider that the "ringform" must be looked upon as an overlapping of the two ends of the nucleus since no true rings are found in human blood though they have been described in the blood of rats. They concede, nevertheless, that in perfect smears and in increased numbers these cells are striking enough to be worthy of consideration as supporting the Arneth theory.

Gruner claims that the "ringform is frequent in carcinoma," that "neutrophils with bizarre forms are characteristic for malignant conditions," that "a relative abundance of lymphocytes is not found in malignant disease," and that "one can produce the blood picture of carcinoma . . . by partaking of certain articles of food, notably pork and to a less extent, other red meats." These statements were corroborated in every respect by my experiments and were, moreover, illustrated in a striking manner by a case of inoperable carcinoma of the sigmoid that will be discussed later on.

My individual cholesterol standard having been established (see Table I), my individual lymphoid defense, its relation to the neutrophil count and the number of ringforms or bizarre forms present in my blood under ordinary conditions, remained to be determined. This was done by a number of differential counts. The blood smears were made before breakfast to eliminate the influence of digestive leucocytosis; 200 cells were counted from 3 smears on every occasion. The variations in the different counts were found to range from 29 to 31 per cent for the lymphoid defense and from 49 to 55 per cent for the neutrophils. The average number of atypical forms in the latter was 2 to 3 per cent of ringforms and 2.5 to 3.5 per cent of bizarre forms. For three consecutive days preceding the experiment the relation was as follows: Lymphoid defense: neutrophils : : 30 : 54; ringforms : bizarre forms : : 2 : 3.

TABLE IV

THE EFFECT OF GRUNER'S DIET ON WRITER'S BLOOD CHOLESTEROL AND CYTOLOGY

EXPERIMENT 4, JUNE 20-23, 1916						
Gruner's diet: Milk and water, lettuce and toast; no butter, but jam <i>ad libitum</i> . Very little jam was used.						
DAY	BLOOD CHOLESTEROL—DUBOSCQ COLORIMETER		LYMPHOID DEFENSE (PER CENT)	NORMAL NEUTROPHILS (PER CENT)	ATYPICAL NEUTROPHILS	
	<i>Bloor I</i>	<i>Bloor II</i>			RINGFORM (PER CENT)	BIZARRE (PER CENT)
	(mg. cholesterol in 6 c.c. chloroform)					
1	0.342	Too brown to test	26	57	2	3
2	0.272	" " " "	30	54	1	3
3	0.186	" " " "	33	46	1.5	3



The slight initial increase observed in the blood cholesterol may have been due to the rather severe nature of the diet to which the body had to become accustomed. On the first day the writer was chronically hungry and had a severe headache in consequence, but during the two following days she felt perfectly well and comfortable. It is a well-known fact that fasting is always accompanied by an increase of the blood cholesterol (Rothschild).<sup>44</sup> The changes found in the cytology of the blood were still very slight, but the drop of the blood cholesterol to 0.186 mg. and the accompanying increase of lymphocytes and decrease of neutrophils is interesting when compared with the results of the subsequent experiments.

TABLE V

THE EFFECT OF AN EXCLUSIVE MEAT DIET ON WRITER'S BLOOD CHOLESTEROL AND CYTOLOGY

EXPERIMENT 5, JUNE 24-JULY 1, 1916						
Exclusive meat diet: Three thin slices of dry bread, 4 by 4 inches, had to be taken after the third day to help the meat down.						
DAY	BLOOD CHOLESTEROL—DUBOSQ COLORIMETER		LMYPHOID DEFENSE (PER CENT)	NORMAL NEUTROPHILS (PER CENT)	ATYPICAL NEUTROPHILS	
	<i>Bloor I</i> (mg. cholesterol in 6 c.c. chloroform)	<i>Bloor II</i>			RINGFORM (PER CENT)	BIZARRE (PER CENT)
1			33	54.5	0.5	1
2			28	65	2.	2
3			24	66	3.	4
4	0.342	0.388	16	72	4.5	9
5			15	73	5.5	5
6	0.358	0.416	19	70	5.5	12
7	0.374*	0.434	18	66	3.5	2
8	0.342	0.458	17	72	9	8.5

\*Diarrhea.

The results obtained by the all-meat diet seem worthy of consideration for several reasons. The changes observed in the cytology and in the cholesterol content of the blood are too marked and too gradual to be explained by mere coincidence. The increase in the blood cholesterol from 0.186 mg. to 0.372 mg. can not be attributed to the effects of hunger because very considerable quantities of meat were consumed at each meal. It is to be regretted that at first the exact amount was not determined by weight, but it may suffice to state that the writer conscientiously tried to eat as much meat as she possibly could for breakfast, luncheon and dinner, a smaller quantity being ingested at 4:00 P. M. It was subsequently found that the average amount of meat consumed daily had been from 1¼ to 1½ pounds. Coffee and tea were taken as usual, but no alcohol, since it is never used. The exclusive meat diet was not accompanied by any unpleasant symptoms whatsoever, although it was found necessary to allow a very small quantity of dry bread after the third day, since meat in hot weather—the experiment was made between June 23 and July 1—loses its attractions as a steady diet. No sensation of hunger was experienced between meals as in the previous experiment, and perfect health as well as unhampered mental activity were enjoyed throughout.\* On the seventh day,

\*Because of its bearing on these experiments I might add that the Union Central Life Insurance Company reported most favorably on the results of their medical examination made at this time.

at the beginning of which the highest cholesterol value was registered, there was a slight attack of diarrhea. On the following day peristalsis was normal again, but the blood cholesterol had dropped to 0.342 per cent. Since the elimination of cholesterol by the feces is one of the means by which the cholesterol balance is preserved (McNee), it seems logical to assume that the body resorted to increased peristalsis in order to maintain its equilibrium of health. The fact that the same automatic regulation of the cholesterol balance was observed also in a later experiment would seem to support this view, and will be more fully discussed in connection with Experiment 7.

The gradual but steady drop in the lymphoid defense from 33 to 17 per cent, the parallel increase of the polymorphoneutrophils from 46 to 72 per cent, and the unusual number of atypical neutrophils—9 per cent ringforms, 12 per cent bizarre—which appeared in the blood as the cholesterol percentage reached its highest values, suggest a very definite relation between the chemical composition and the cytology of the blood, while the changes observed in both appear to be closely associated with the chemical nature of the food consumed. The same observations could be made in every one of my experiments.

The cytologic changes found in the blood during the exclusive meat diet are moreover in perfect accordance with Gruner's findings, which they corroborate. The two points on which he lays particular stress regarding the similarity between the blood picture during excessive meat consumption and in carcinoma—reduction of the lymphocytes and the appearance of atypical neutrophils in markedly increased numbers—seem to be borne out also by the following observations:

OBSERVATIONS A AND B

SUBJECT	BLOOD CHOLESTEROL		LYMPHOID DEFENSE (PER CENT)	NEUTROPHIL COUNT (PER CENT)	ATYPICAL NEUTROPHILS (RINGFORM) (PER CENT)
	DUBOSQ Bloor I	COLORIMETER Bloor II (mg. cholesterol in 6 c.c. chloroform)			
A. Dr. F.: Inoperable carcinoma of sigmoid .....	0.416	0.416	11	70	10
B. Man in perfect health, who had tropical malaria sixteen years ago	0.462	0.472	40	50	2

It is noteworthy that whereas the blood cholesterol of the healthy person is slightly higher than that of the carcinomatous patient, the unusually high lymphoid defense, 40 per cent, and the small percentage of ringforms, 2 per cent, of the former are in striking contrast to the unusually low lymphoid defense, only 11 per cent, and the high percentage of atypical neutrophils, 10 per cent, of the latter.

The relation between the blood cholesterol and the blood cytology is further illustrated by the changes produced in the blood of the same carcinomatous patient, Dr. F., by radium therapy, which temporarily arrested the disease if it did not cure it, and caused a marked improvement in the general condition.

## OBSERVATIONS C AND D

SUBJECT	BLOOD CHOLESTEROL		LYMPHOID DEFENSE (PER CENT)	NEUTROPHIL COUNT (PER CENT)	ATYPICAL NEUTROPHILS	
	DUBOSCQ	COLORIMETER			RINGFORMS	BIZARRE
	<i>Bloor I</i>	<i>Bloor II</i>			(PER CENT)	
	<i>(mg. cholesterol in 6 c.c. chloroform)</i>					
C. Dr. F. after colostomy . . . . .	0.330	0.374	21.5	67	3	7
D. Dr. F. after radium treatment.	0.240	0.260	33	62.5	3.5	0

During the following months the lymphoid defense of the patient dropped again to 16 per cent, which seems in accordance with the view expressed by the patient's physician, Dr. Howard Kelly, that "a permanent cure was not to be expected." It is greatly to be regretted that the blood cholesterol could not be tested again, since there is every reason to assume that a renewed increase in the blood cholesterol would have corroborated previous observations. Although at present definite conclusions would be premature, there seems little doubt that further investigations of the relation between the chemistry and the cytology of the blood may prove to be of great value in the study of malignant conditions. In the meantime the explanation seems admissible that a high lymphoid defense is effective in maintaining the normal equilibrium even in the presence of high cholesterol values, although other factors, elimination of cholesterol by the feces for instance, may be equally important. Levy and Rowntree have shown that the balance of health, that is, the chemical balance of the body, swings between extremely narrow limits. "The acidity of 'acidosis' and the alkalinity of 'alkalosis' may be compared with perfect scientific accuracy to the 'acidity' of distilled water and the alkalinity of certain varieties of tapwater" (Rowntree). Consequently, any factors by which the normal equilibrium might be disturbed are worthy of consideration. The cholesterol content of the blood as well as the lymphoid defense would appear to be factors of no small importance.

## VEGETABLE DIET

The diet used in this experiment consisted of vegetables, fruit, and dry bread or toast. No butter was used except such as had been added to the vegetables in cooking, and since the latter were not very "rich"—never creamed for instance—the amount of butter consumed can be safely considered very small. The vegetables consisted of carrots, cauliflower, string beans, asparagus, boiled or mashed potatoes, spinach, lettuce and cucumbers. In a few instances, mushrooms were not avoided, but the steady decrease of the blood cholesterol (below normal) would tend to show that their consumption did not seriously interfere with the results of the experiment even though their cholesterol content is relatively high. No fish and no meat in any form were partaken of during the time of experimentation. Coffee and tea were drunk as usual, but no alcohol in any form. It would seem that the diet was entirely sufficient to meet the requirements of the body, for the duration of the experiment at least, since no sensation of hunger between meals was experienced and no loss of weight could be observed. Peristalsis was absolutely normal and the writer felt extremely well and "fit" throughout the experiment.

TABLE VI

## THE EFFECT OF A VEGETABLE DIET ON WRITER'S BLOOD CHOLESTEROL AND CYTOLOGY

EXPERIMENT 6, AUG. 2 TO 12, 1916						
DAY	BLOOD CHOLESTEROL DUBOSCQ COLORIMETER (mg. cholesterol in 6 c.c. chloroform)		LYMPHOID DEFENSE	NEUTROPHIL COUNT	ATYPICAL NEUTROPHILS RINGFORM BIZARRE	
1			17	71	3.5	3
2	0.312	0.386	28	65	0.0	3
3						
4	0.302	0.358	21	70	0.5	3.5
5			33	58	1	1.5
6	0.202	0.312	28	60	2.5	0.5
7						
8	0.242	0.312	30	61	1.5	0.5
9	0.242	0.302	29	62	1	2
10	0.242	0.302	33	57	0.5	3

The results obtained by the vegetable and fruit diet present a contrast as well as a complement to those of the exclusive meat diet. Whereas the blood cholesterol slowly but steadily decreased from 0.342 mg. to 0.202 mg. on the fifth day and finally seemed to settle down to a new standard, 0.242 mg., which was found on three consecutive days, the lymphoid defense increased from 17 per cent to 33 per cent, with a parallel drop in the neutrophils and a marked reduction of the number of atypical cells.

In order to give the body sufficient time to swing back to normal values, and because of the tendency to collapse shown by the cubital veins, described above, eleven days were allowed to elapse before another blood test was made. During this interval the usual mixed diet was used again. The following values were found at its close:

BLOOD CHOLESTEROL DUBOSCQ COLORIMETER <i>Bl or I</i> <i>Bloor II</i> (mg. cholesterol in 6 c.c. chloroform)		LYMPHOID DEFENSE (PER CENT)	NEUTROPHIL COUNT (PER CENT)	ATYPICAL NEUTROPHILS RINGFORM BIZARRE (PER CENT) (PER CENT)	
0.266	0.307	31	62	0.5	3

Apart from the very slight increase in the neutrophil count, the normal standard had been reached again. The difference between the original cholesterol standard, 0.272 mg., and the last reading, 0.266 mg., i. e., 0.006 mg., can hardly be considered significant. The intimate relation between the chemistry and the cytology of the blood and the diet, previously observed, seems to be confirmed by this experiment.

INFLUENCE OF AN EXCESS OF CARBOHYDRATES ON THE CHOLESTEROL CONTENT  
AND THE CYTOLOGY OF THE BLOOD

Two seemingly unrelated problems formed the basis of this experiment; namely, the cause of the epithelial proliferation on the tongues of rats observed by Stahr<sup>55</sup> after exclusive feeding of oats, and the ability of the body cells to synthesize cholesterol from cholesterol-free substances, defended by Dezani.<sup>12, 13</sup>



The practical importance of these problems and their possible relation to each other is such that a brief outline of the work referred to may be of value.

Stahr found that epithelial tumors of considerable size on the tongue originating without exception from the papillae circumvalate could be produced in a great number of rats of different species by feeding the animals exclusively with oats. No other diet resulted in this tumor formation. Congenital tumors were never observed, although as many as seven generations of the same family were used in the experiment. The best results were obtained when the animals were fed on oats continually and almost exclusively ("dauernd und fast ausschliesslich") as soon as they had been weaned. In very old rats the excessive oat diet did not seem to cause any epithelial proliferation, while adult rats, that is, full grown but not old, animals seemed to respond to the irritant in varying degrees. Stahr attributes his results, in part at least, to chronic irritation produced by the oat husks which were found embedded in the tongues of the animals in which tumors occurred. Nevertheless, he admits that a number of wild rats in whose tongues the sharp pointed husks were also found embedded showed no trace of tumor formation. The time required for the lesions to become manifest and their respective size varied considerably in different animals. A great number of the larger tumors developed in from five to seven months, the initial stages being visible in about four weeks to two months. In many instances, however, the tumors grew so slowly that there seemed to be no visible increase after five months. Stahr considers that this divergence can be accounted for only by the fact that the rats were not endowed with the same degree of predisposition ("dass die Ratten eben verschieden stark disponiert waren"), a conclusion which will readily be admitted. He adds that our conception of the nature of this so-called "disposition und konstitution," which has been somewhat vague for many years has become clear and definite as a result of recent investigations. The accuracy of the latter statement seems questionable. That the mechanical factors which he enumerates—*anatomical formation of the mouth and motility of the tongue, either of which impeded or promoted the removal of the implanted husks, the nature of the irritant itself and the length of time the irritation was kept up*—played an important part in the process of atypical proliferation can not be doubted, but there are other factors the significance of which should not be underrated; namely, the chemical composition of the oat diet and the power of elimination of the individual rat. Notwithstanding the masterly analysis of causes found in his report, Stahr does not seem to take these factors into consideration, for the internal causes (*innere Ursachen*) which he discusses at great length are of an entirely different character. That both the "disposition and constitution" to which he refers, however, are intimately associated with the ability of the individual organism to eliminate or metabolize certain articles of food, will, I think, be readily conceded. Many of the problems of individual metabolism have by no means been solved as yet.

In discussing the nature of the growths produced by the oat diet, Stahr states that they represent "at least the earliest evidence of an epithelial tumor, of a true blastoma,"<sup>55</sup> (page 225). He discusses the relation between harmless proliferation and malignant hyperplasia at great length and comes to the con-

clusion that in the initial stages it is impossible to decide whether any given type of atypical epithelial proliferation is destined to retain its benign character or to develop into a true carcinoma, "so that consideration must be taken of internal causes which affect these originally benign growths in such a way that they discard normal bounds and develop into cancer." The context shows that Stahr evidently looks for the "internal causes" within the tumor cells themselves, inasmuch as he defends the hypothesis that in malignant conditions new types of cells are formed in circumscribed areas, and lawless proliferation is an inherent characteristic of these newly formed cells. In other words, the tumor cells as such are responsible for their onslaught on the welfare of the body. Would it not be equally reasonable to assume that lawless proliferation is the manifestation of "internal causes" in another sense; that is, that some substance stimulating cell proliferation is supplied by the body itself through faulty metabolism, and that the atypical character of these proliferating cells is merely the result of an over-hurried rate of cell-production under the influence of this constantly applied stimulant? We know that in hyperthyroidism, for instance, atoxic substance is constantly produced which goads the heart into lawless activity, and that infinitesimal doses of this substance, Kendall's<sup>26</sup> alpha iodine compound, similarly increase the heart rate of normal animals for a short time. This has been demonstrated experimentally. It has also been found that the normal heart swings back to its usual rate of action as soon as the toxic compound has been either eliminated or destroyed by normal metabolism. We admit the "unripe" character of the so-called malignant cells and we know that the more embryonic or unfinished the cells of a tumor are, the more rapid and destructive its growth. Would it not be reasonable to deduce from the foregoing facts, that substances supplied in the food and insufficiently metabolized by inadequate organs could become the cause of lawless cell proliferation, inasmuch as the daily intake of food would furnish a constant stimulant that might bring about the hurried coinage of unfinished, atypical cells, embryonic in character, simply because the rate of production did not allow them time to become full-grown? It is well known that under suitable conditions normal cells can be transplanted and made to grow *in vitro* (Foote<sup>16</sup> and Burrows). The origin of metastatic tumors can be traced in many instances to strands of single cells that have been crowded out by the rapid growth of the mother tumor, and the danger of scattering and implanting stray tumor cells in operative procedures is recognized by surgeons. These observations, as well as many others that can not all be enumerated here, seem to support the view that the body itself furnishes the conditions by which proliferation is either regulated and kept within normal bounds or incited to become lawless and destructive. That the invasive growth of tumor cells ("infiltratives Wachstum," Joest and Ernesti) might be interpreted as merely the result of the increased rate of production referred to above, does not seem inadmissible.

The fact that the majority of the oat-fed rats developed tumors, the striking absence of tumors in animals fed on other food, and the presence of oat husks in the tongues of wild rats that showed no tendency to tumor formation (proving that the irritant alone was insufficient to produce the lesions) would seem to indicate that the diet itself and the way in which the animal was able to handle it,

may have been primary factors in the results obtained by Stahr. The chemical analysis of the blood of these tumor rats and the study of their blood cytology would undoubtedly have furnished valuable data concerning the effect of the diet on the development of the tumors.

The synthesis of cholesterol by the body cells from substances containing only the elements carbon, hydrogen and oxygen, of which the cholesterol molecule,  $C_{27}H_{46}O$ , is composed, has been a subject of much controversy. Bloor<sup>6</sup> (page 581) makes the guarded statement that together with other lipoids, cholesterol is "probably" synthesized by the animal organism, whereas Rothschild<sup>41</sup> (page 233) boldly asserts that "there is no synthesis of cholesterol in the body." In discussing the question, Bloor calls attention to the work of Dezani,<sup>12, 13</sup> Gardner and Lander. While the experiments of Gardner and Lander furnish a striking illustration of the power possessed by the animal organism to transform cholesterol into body cells, or at any rate, to utilize it for the purpose of cell formation, the term "synthesis" would hardly seem applicable to the process they studied; that is, the gradual development of the chick embryo from the egg. Synthesis may be defined as "the artificial building up of a chemical compound by union of its elements." Since the egg already contains a high percentage of cholesterol, the growing chick does not need to effect the union of the elements but merely converts the substance into cells. Moreover, "the fully developed chick contains only as much cholesterol as the egg did before hatching" (Rothschild,<sup>41</sup> page 230).

Dezani's work, on the other hand, seems far more suited to demonstrate a true synthesis of cholesterol, as a brief abstract of his original report will show. Having established the average weight and the average total cholesterol content of a certain breed of mice, he divided the sixteen remaining animals into groups of four. These animals were fed for twenty-three days on a mixture of casein and maize from which all the cholesterol had been extracted for a week with ether-alcohol. In addition, Group I received a certain amount of mineral salts, Group II mineral salts and lecithin, Group III mineral salts and fat in the form of fatty acids and glycerin, and Group IV mineral salts, lecithin and fat in the same form. The mice devoured this food greedily and seemed to thrive on it. Young growing mice had been chosen for the experiment. They gained in weight, developed normally and seemed in perfect health. At the end of seventeen days however, they began to dislike the food and lost slightly in weight. Their coats became rough, but they were lively and the ears showed no sign of anemia. On the twenty-third day, three of the mice were found dead in their cage and the rest were killed. The total cholesterol of each group was then determined by desiccating the bodies *in toto* and extracting the cholesterol by means of a special procedure devised by Dezani. Though no cholesterol had been supplied in the food, chemical analysis had shown that cholesterol had been daily eliminated by the feces, and the postmortem determinations proved that the total average cholesterol had been increased by about one-third in each group. The mice had been weighed in groups, and every group exceeded its initial weight, whereas a slight loss of weight which occurred during the last days of the experiment did not exceed 0.8 per cent of the total gain. Dezani considers, therefore, that the



destruction of body cells alone can not account for the marked increase of the total cholesterol, though it may have added slightly to the values, and that the animals must have synthesized the greater part of the surplus cholesterol from the cholesterol-free food on which they had been fed. He suggests the following explanation:

Under ordinary circumstances the body does not synthesize cholesterol, the amount essential to health being easily obtained "ready-made" in the food. Under special conditions, however, when no cholesterol is available, synthesis is resorted to by the body as an extreme measure in preference to the alternative of a cholesterol deficit. In other words, the body can synthesize cholesterol under the stress of necessity just as the heart can use all its reserve power when occasion demands. The organism soon tires of the unwonted effort, however, and loss of health, or decompensation, results in the end, as was the case with Dezani's mice.

The question suggested by Stahr's experiment is the following: Could the results obtained have been due to a reduction of the lymphoid defense with parallel increase of the blood cholesterol in those animals which responded to the excessive oat diet by tumor formation? The relation between the blood cholesterol, the lymphoid defense, and the diet observed in the writer's experiments and the part played by cholesterol in cell proliferation both in normal growth and in the growth of tumors (Robertson and Burnett), justify this supposition, especially as Stahr's report contains no reference to the blood cytology or blood chemistry of his animals.

No data being available in regard to the cholesterol content of oats, and the only chemical analysis of any kind of grain recorded being that of maize with 0.10 per cent of cholesterol (see Table I), it was decided to make a test by the Bloor method of the broken oats sold under the name of "Scotch oatmeal."\* This test was entirely negative. A second test was made according to the Autenrieth method on the assumption that the Bloor test might not be effective in dissociating certain chemical compounds in which the cholesterol in oats might be bound up, though as Mueller<sup>33</sup> has pointed out, Bloor's method of extraction "will be found to be practically complete" in other substances. By Autenrieth's method the substance to be tested is boiled for two hours with 25 per cent potassium hydroxide, and the combined action of strong alkali and boiling could reasonably be expected to effect the necessary dissociation if any cholesterol were contained in the oats in compound form. However, the results of this test were negative also. Since, then, no cholesterol appeared to be contained in oats, and Dezani's work suggested that even cholesterol-free food can cause an increase of the total cholesterol content of the body by synthesis of cholesterol, it still seemed possible that Stahr's rats had synthesized their cholesterol from the cholesterol-free oats. This surmise did not seem unreasonable because, as they are carbohydrates, oats contain the very elements, carbon, hydrogen, and oxygen, of which the cholesterol molecule is composed.

In order to verify this supposition a diet consisting as exclusively as possible

\*Scotch oatmeal consists entirely of broken oats, and should not be confounded with the so-called "rolled oats."



of broken oats (Scotch oatmeal) was decided upon. Such a diet, even though the husks had been removed from the grain, would contain practically the same chemical compounds as the oats on which Stahr's rats had been fed. The chemical effects of the diet on the cholesterol content and the cytology of the blood being the chief object of investigation, the absence of the oat husks did not seem of great practical importance. That no vitamins were lost by the removal of the oat husks would seem to be demonstrated by Fig. 5, which shows that the oat grains were practically intact and surrounded by a slightly hairy capsule, thus being comparable to thrashed but unpolished rice.

Although for reasons to be discussed later, this experiment did not furnish conclusive data concerning the synthesis of cholesterol in the body, the results obtained may be of value inasmuch as they again confirm previous observations regarding the intimate relation between the diet, the lymphoid defense, and the cholesterol content of the blood.

Rothschild<sup>44</sup> has pointed out that the cholesterol content of the blood is increased by starvation, cholesterol being liberated from the body fat in which it is stored. Loss of weight was, therefore, to be studiously avoided during the oatmeal diet. It might be argued that the maintenance of the original body weight and especially an increase thereof in the course of the experiment would be ample proof that any rise in the blood cholesterol could not be attributed to the destruction of body tissues. However, Dezani's<sup>12</sup> first experiment had shown that the body will make every effort within its power to maintain its cholesterol balance even during starvation, and that a loss of 41 per cent of body weight is accompanied by a loss of only 15 per cent of the total cholesterol. Consequently, it seemed safer not to depend on the evidence of body weight alone, but to base the experiment on a strict maintenance of the metabolic balance in general. The following standards were therefore established:

1. The diet must consist of ground oats (Scotch oatmeal) as exclusively as possible.

2. It must contain all the elements needed to maintain the metabolic balance; namely, the requisite number of calories, the relative amounts of protein, fat, and carbohydrates.

3. The food requirements, compiled from Friedenwald and Ruhräh's<sup>18</sup> (pages 48-79) data corresponding to the age, sex and occupation of the experimenter, must be met, if necessary, by additional foodstuffs containing as little cholesterol as possible.

According to Voit, Rubner, Tigerstedt, Atwater and Benedict,<sup>18</sup> quoted by Ruhräh,<sup>18</sup> (pages 51-54), the daily food requirements of the male adult doing light and moderately light work are as follows:

	CALORIES	PROTEIN GM.	FAT GM.	CARBOHYDRATES GM.
Voit: Physician at moderate work,	2,833	127	89	362
Rubner: Male adult at moderate work,	2,600			
Tigerstedt: Shoemaker at moderate work,	2,001-2,400			
Atwater: German physician,	2,680	131	95	327
Japanese professor,	2,380	123	21	416

Friedenwald and Ruhräh<sup>18</sup> (page 69) suggest the following standard:

For light work, 17 calories per pound of body weight.  
For moderate work, 20 calories per pound of body weight.

They state further (pages 55-56) that "curiously enough, mental work does not apparently utilize heat or energy in the ordinary way. . . . In a respiration colorimeter, hard mental work; i. e., the working out of abstruse mathematical problems requiring hours of time, does not cause any difference in registration. The same apparatus, however, is sufficiently sensitive to register the heat generated by turning over in bed or by raising an arm." In discussing the relation of sex and body weight to food values required, they state<sup>18</sup> (page 57): "On an average, women are only about four-fifths as large as men, and consequently dietaries for groups of women will require four-fifths the amount of food."

In consideration of these data and since carbohydrates were to form the chief component of the oatmeal diet, the food requirement of the Japanese professor given by Atwater, divided by four-fifths, were used as the basis for the oatmeal experiment.

EXPERIMENTER	OCCUPATION	CALORIES	FOOD REQUIREMENTS		
			PROTEIN GM.	FAT GM.	CARBOHYDRATES GM.
40 years old; Weight, 134.5 lb.	Laboratory research, Mental, light manual,	2,000	100	30	325

The amount of fat was increased somewhat because the climate is rigorous, the experiment being made at the beginning of the cold season between October 26 and November 1, and because a liberal margin of fat in the diet might also guard against the combustion of the body fat. Moreover, the values quoted above would coincide satisfactorily with Ruhräh's estimate of the food requirements of a woman weighing 134.5 pounds at light work—17 (calories per pound of body weight, man at light work)  $\times$  134.5 (pounds body weight)  $\div$   $\frac{4}{5}$ —and the relative caloric values given by him for protein, fat and carbohydrates:

1 gram protein	= 4 calories	100 grams protein	= 400 calories
1 " fat	= 9 "	30 " fat	= 270 "
1 " carbohydrates	= 4 "	332 " carbohydrates	= 1328 "
			Total.....1998 "

From the figures given by Friedenwald and Ruhräh<sup>18</sup> (page 49), the fuel value of oatmeal and its relative percentage of protein, fat, and carbohydrates may be calculated as follows:

	CALORIES	PROTEIN	FAT	CARBOHYDRATES
Oatmeal (raw) 1 lb.	1800*	67 gm. (15%)	22 gm. (5%)	360 gm. (80%)

\*Rolled oats = 1850.

Consequently the consumption of one pound of oatmeal would almost suffice to meet the food requirements, the slight deficit in the calories and protein being easily balanced by the addition of a small amount of other food with low cholesterol content, while the surplus of carbohydrates (28 gm.) contained in the oatmeal made up the deficit in fat. (2.5 gm. of carbohydrate being equivalent to 1 gm. of fat.)

An unexpected difficulty presented itself, however, in the process of cooking, owing to the amount of water absorbed by the cereal, the original bulk of oatmeal increases no less than four times. As a result, the consumption of a whole pound of oatmeal in its magnified form proved to be a physical impossibility, defying the most determined efforts. A compromise had to be resorted to and the following diet was adopted:

	CALORIES	PROTEIN	FAT	CARBOHYDRATES
		GM.	GM.	GM.
Oatmeal, $\frac{3}{4}$ lb. (raw weight)	1200	48	16	240
Oranges, 3	180	0	0	18
Milk, 2 glasses	320	14	24	30
Sugar, $\frac{1}{4}$ lb.	450	0	0	112
Dried beef, $\frac{1}{8}$ lb.	66	16	3	0
	2216	78	33	400

In this combination, the total minimum requirement in calories was slightly exceeded, while the carbohydrate surplus of 100 gm. amply made up for the protein deficit of 22 gm., since according to Rubner, 322 gm. of carbohydrates represent a fuel value equivalent to 211 gm. of protein. The diet seemed to guarantee a perfect maintenance of the metabolic balance, whereas the amount of cholesterol contained in 2 glasses of milk and  $\frac{1}{8}$  lb. of dried beef (the latter chosen on account of the salts and relatively small bulk) was far below that provided in eggs, butter, and meat during the ordinary mixed diet. (Milk 0.080 mg. cholesterol; dried beef 0.181 mg. cholesterol. (See Table II.)

The following results were obtained on the six days of oatmeal diet: Within three days the blood cholesterol rose from 0.266 mg. to 0.400 mg.; simultaneously the lymphoid defense dropped from 31 to 24 per cent (Table VII). As in previous experiments, the cytology of the blood kept pace with these changes, for as the blood cholesterol reached its maximum and the lymphoid defense its minimum, the ringform and bizarre forms of neutrophil leucocytes increased from 2 and 3 per cent respectively to 8 and 12 per cent.\* A steady increase in weight accompanied the above changes, showing that they were in no wise due to insufficient nutrition. On the fifth day of the experiment there was a sudden rise in the lymphocyte count from 24 to 31 per cent, preceded by a peculiar sharp pain in the left side the day before. This pain was confined to the region of the spleen. It differed from the pain caused by flatulence inasmuch as starting at 4 o'clock P. M., it never shifted or abated until about 11:30 P. M. when the experimenter fell asleep. During the two following days the splenic region remained very sore but the pain was no longer acute. The fact that the sudden increase in the lymphocyte count was preceded by pain in the region of the spleen suggests that an increased activity of that organ may have been accompanied by a certain amount of swelling. The pain itself was caused in all probability by adhesions around the spleen due to attacks of "Dutch malaria"† from which the writer suffered for

\*It will be remembered, that all the data, blood counts, cholesterol values, and weight were taken before breakfast throughout these experiments and that consequently digestive leucocytosis can be eliminated as a cause of the sudden change in the lymphocyte count.

†"Dutch malaria" is clinically known as recurrent fever. The cause of the illness, the spirilla obermeieri, was found in the writer's blood at the time. Although the disease can be differentiated bacteriologically from other types of malaria, the subjective symptoms are practically identical. The spleen and the liver are often found enlarged, but these findings are by no means constant, and they did not occur in the writer's case. During one attack, however, a severe throbbing pain was felt in the splenic region and lasted for several weeks. This may have been caused by perisplenitis or by a splenic abscess. Since in the absence of a marked enlargement of the spleen neither of these conditions can be diagnosed with certainty in the living, the autopsy report alone can give conclusive evidence.

TABLE VII  
THE EFFECT OF AN OATMEAL DIET AND A MIXED DIET ON WRITER'S BLOOD CHOLESTEROL AND CYTOLOGY

DAY	DATE	FOOD	WEIGHT (LB.)	EXPERIMENT 7, OCT. 27 TO NOV. 8, 1916				REMARKS	
				BLOOD CHOLESTEROL DUBOSQ COLORIMETER	LYMPHOID DEFENSE	NORMAL NEUTROPHILS	ATYPICAL NEUTROPHILS RINGFORM		
				( <i>mg. cholesterol in 6 c.c. chleform</i> )	(PER CENT)	(PER CENT)	(PER CENT)		
1	Oct. 27	Oatmeal diet	134.5	0.266	31	61	3	4	Mixed diet since August; blood taken before breakfast.
2	28	"	135	0.333	32.5	54.5	5.5	4	Slight discomfort from flatulence; bowels regular.
3	29	"	135						Much discomfort from flatulence, otherwise well. Bowels regular, but stool deep yellow; no diarrhoea.
4	30	"	135.5	0.400	24	65.5	6.5	5	Flatulence very pronounced from 4 P.M. till asleep, 11 P.M.; pain in spleen; * bowels and stool like day before.
5	31	"	136	0.420	31	62	6	5	Flatulence pronounced from 4 P.M. on; spleen region sore; slight diarrhoea.
6	Nov. 1	"	136	0.233	26.5	62.5	6	7	Flatulence from 4 P.M. very pronounced; spleen pain slight; slight diarrhoea.
1	2**	Mixed diet	136.5	0.266	31	58	3.5	3	No flatulence; bowels regular; stool deep yellow; no spleen pain; fagged; otherwise well.
2	3	"	136	0.266	36	57	1.5	2	Feeling very well; no flatulence; bowels regular.
3	4	"	135.5	0.295	27	62	5	2.5	Very well; bowels regular; stools deep yellow.
4	5	"	135	0.347	26	64			Feeling very well but slight diarrhoea.
5	6	"	135	0.380	30	55			Feeling very well; bowels regular; stools normal.
6	7	"	134.5	0.266	30	53			Feeling well; bowels regular; stools normal.
7	8	"	134.5	0.266	30	53			

\*The pain in the region of the spleen did not shift at all, thereby proving that it was not gas pain due to flatulence, as seemed probable at first. The possible origin of this pain is considered in the discussion of the experiment.

\*\*From Nov. 1 to 2 only  $\frac{1}{2}$  lb. of oatmeal and  $\frac{1}{8}$  lb. of sugar were consumed, owing to the fact that the writer attended a dinner party. The low value of the blood cholesterol on the following morning was probably due to increased peristalsis during the previous day, and seems to indicate that the small amount of meat eaten at the party was not sufficient to increase the cholesterol value.

\*\*\*Slides thrown away by mistake so that the average could not be established as on the preceding days by counts made by three different workers whose results tallied with only very slight differences.



several years, but of which no recurrence had been observed during the past six years.

The delicate and automatic self-adjustment by which the normal balance is regulated in health is again illustrated by this experiment. After the blood cholesterol had reached the maximum of 0.400 mg., the lymphocytic reaction alone seems to have been unable to restore the balance and the blood cholesterol was again reduced by increased peristalsis as in the "all meat" diet. The cause of the increased peristalsis will be considered in the discussion. As soon as the ordinary mixed diet was used the increase in weight, from 134.5 lb. to 136.5 lb., also disappeared and the normal weight, 134.5 lb., which had been constant for several years, was again registered within six days. The gradual loss of weight increase was accompanied by a slight rise of the blood cholesterol (0.266 mg., 0.295 mg., 0.347 mg.), showing, that although the increase in weight may have been due in part to the absorption of water from the oatmeal diet by the tissues, a certain amount of fat had also been produced, as reduction of fat is accompanied by a rise in the blood cholesterol (Aschoff, Rothschild). After the fifth and sixth days, the normal cholesterol value, 0.266 mg., remained constant. The lymphoid defense was still slightly below the figure observed at the beginning of the experiment, but the numbers of atypical neutrophils, ringforms and bizarre forms had returned to 3.5 and 3 per cent respectively.

At the time of writing, Dec. 30, 1916, the blood cholesterol, lymphoid defense, atypical neutrophils and weight are as follows, the usual diet having been eaten since the end of the experiment: Blood cholesterol 0.275 mg.; lymphoid defense, 32 per cent; atypical neutrophils, ringforms, 1.5 per cent, bizarre forms, 2.5 per cent; weight, 135.0 lb. These figures seem to support the deduction that, under normal conditions there is a constant relation between the above named factors.

#### DISCUSSION

Whereas the influence of the diet on the blood cholesterol and the cytology of the blood seems sufficiently clear in our experiments to require no further comment, three other points must be briefly discussed; namely, the cause of the increase in the blood cholesterol during the oatmeal diet which contained less cholesterol than the ordinary mixed diet, the cause of the slight attacks of diarrhea, which occurred whenever the cholesterol values reached their maximum, and the significance of the atypical neutrophils.

It has already been stated that the increase of the blood cholesterol in the oatmeal experiment did not furnish conclusive evidence as regards the synthesis of cholesterol from cholesterol-free food, even though Dezan's work seemed to prove that cholesterol can be synthesized under special conditions. The lack of definite proof in our case may be readily explained by the fact that the methods used by Dezan for the determination of the total cholesterol of his mice could not be applied in the writer's work for obvious reasons. As the increase of the cholesterol values could be ascertained in the writer's experiments only by means

of small blood samples, the possibility remained that cholesterol stored in various organs, the adrenals and the liver, for instance, had been mobilized in some way by the diet, and that the increase was due to a mobilization rather than to an actual synthesis. Although this deduction seemed plausible, it could not be verified, of course, and the rise of the blood cholesterol on a cholesterol-poor food was by no means explained. Mueller's<sup>34</sup> recent investigations may furnish a satisfactory explanation, however, inasmuch as he was able to show that the pancreas plays a hitherto unsuspected part in cholesterol metabolism. Although it had been known for some time that high cholesterol values are by no means rare, if not constant, in diabetes, the observation had not been accounted for so far as the writer is aware. Mueller<sup>34</sup> analyzed the gastroduodenal content of dogs after cholesterol feeding and studied cholesterol absorption in the digestive tract after elimination of the bile and pancreatic secretion (biliary fistula, ligation or resection of the pancreatic duct. He also studied the effect of the digestive enzymes on cholesterol *in vitro* and found that the pancreatic secretion was needed for the formation of cholesterol esters; that no cholesterol could be absorbed by the intestine without esterification; that no cholesterol absorption took place after ligation of the pancreatic duct; and that biliary fistulas lessened, but did not prevent the absorption of cholesterol, (the latter observation is in contradiction to Rothschild's findings),<sup>41</sup> but that the formation of cholesterol esters was markedly accelerated by the combined action of the bile and the pancreatic juice.

The bearing of Mueller's investigations on our experiment appear to be as follows: It seems possible that as the amount of carbohydrates provided in the oatmeal diet was relatively greater than the amount usually consumed in the mixed diet, (though it did not greatly exceed the average carbohydrate requirements suggested by Friedenwald and Ruhrah), the increased demands on carbohydrate metabolism, and especially the sudden change of diet, may have somewhat overtaxed the pancreas. If this deduction be correct, the greater part of the pancreatic secretion may have been used to metabolize the sudden increase of carbohydrates and but little of its activity could have been devoted to the esterification of the body cholesterol. Since free (nonesterized) cholesterol can not be absorbed by the intestinal mucosa, it would have remained in the circulation, causing a rise in the blood cholesterol. It is to be regretted that Mueller's<sup>34</sup> publication was not available at the time of our experiment and that consequently the effect of the oatmeal diet on the pancreas was not taken into consideration. Although urinalysis had given normal results both during the oatmeal diet and in the previous experiments, it is possible that blood-sugar determinations might have revealed a slight degree of glykemia and thus also have corroborated the above deduction.

The slight attacks of diarrhea which occurred in the experiments here reported whenever the blood cholesterol reached a given maximum, have been interpreted by the writer as an evidence of the automatic regulation of the cholesterol balance. This interpretation may be defended on the following grounds: Although an exclusive diet of fruit and vegetables is known to stimulate the activity of the intestinal mucosa and to increase peristalsis, the bowel

movements were absolutely normal during the experimental fruit and vegetable diet. At the same time, the blood cholesterol dropped below normal whereas the lymphoid defense was only slightly higher than usual. The writer's observations on the prompt readjustment of the cholesterol values after digestion also suggest that a healthy organism has natural means at its disposal by which it can and does prevent the accumulation of cholesterol in the blood. In relation

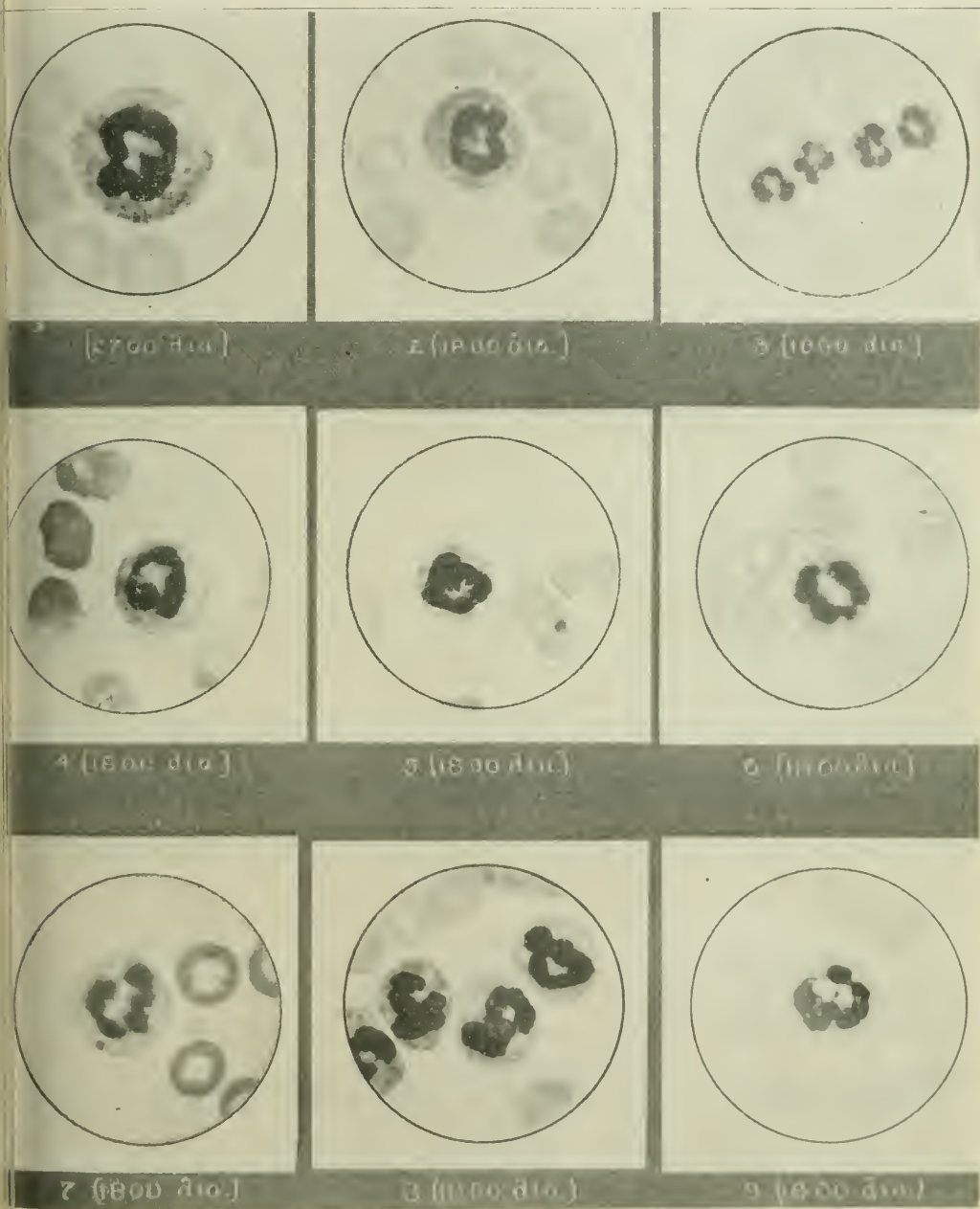


Fig. 1.—Atypical neutrophils in the blood in carcinoma of the sigmoid: Ringforms.



to this normal automatic regulation of the cholesterol balance, as well as the increase of the blood cholesterol found in a great many carcinoma cases, and the effect of cholesterol on cell proliferation referred to at the beginning of this paper, the almost constant reference to "chronic constipation alternating with diarrhea" which the writer has observed in the clinical histories of a great

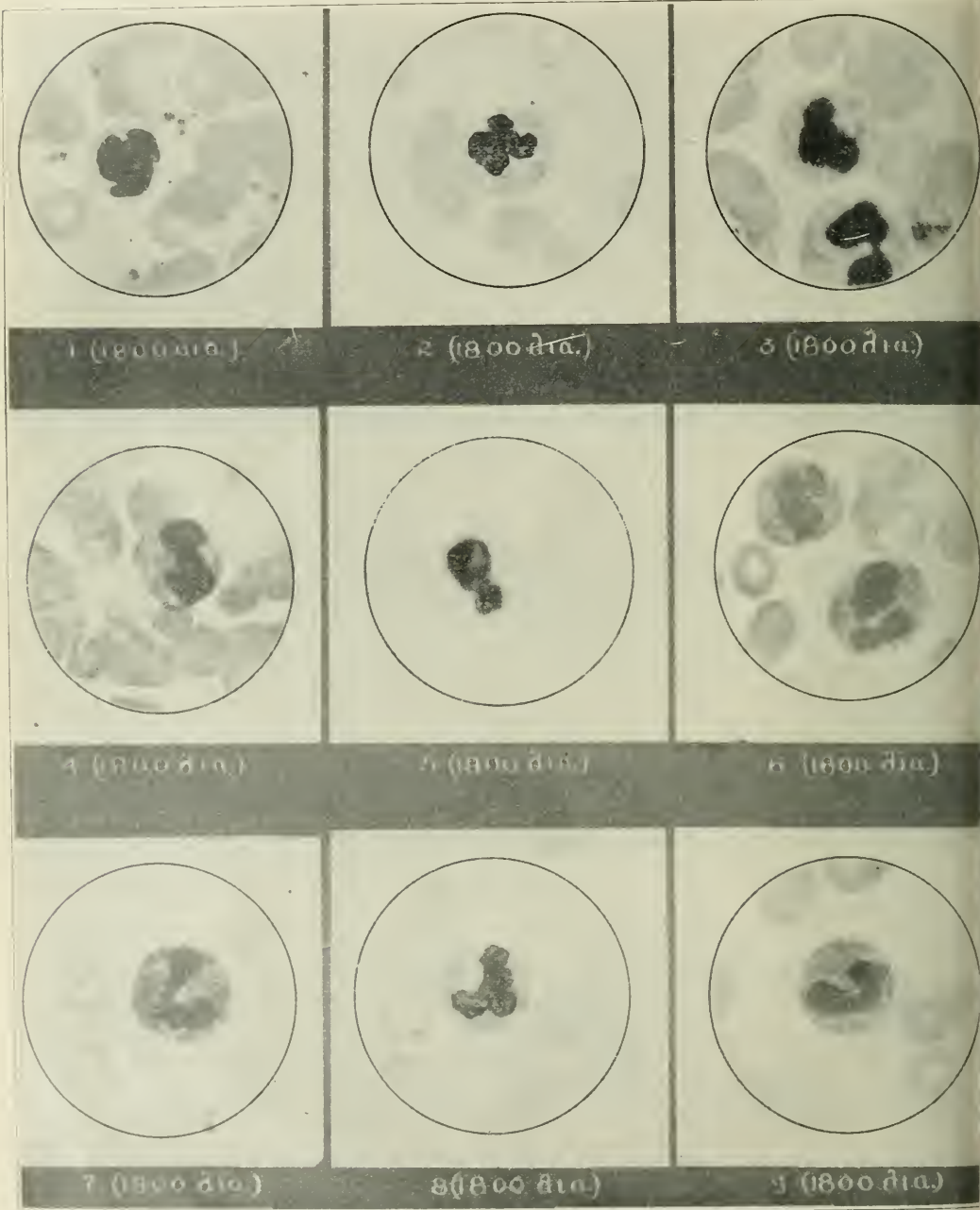


Fig. 2.—Atypical neutrophils in carcinoma of the sigmoid: Bizarre forms.



number of patients suffering from various types of cancer, may not be without significance.

The significance of the atypical neutrophils is still a matter of speculation. Their unusual, often fantastic, morphology seems to make them worth consider-

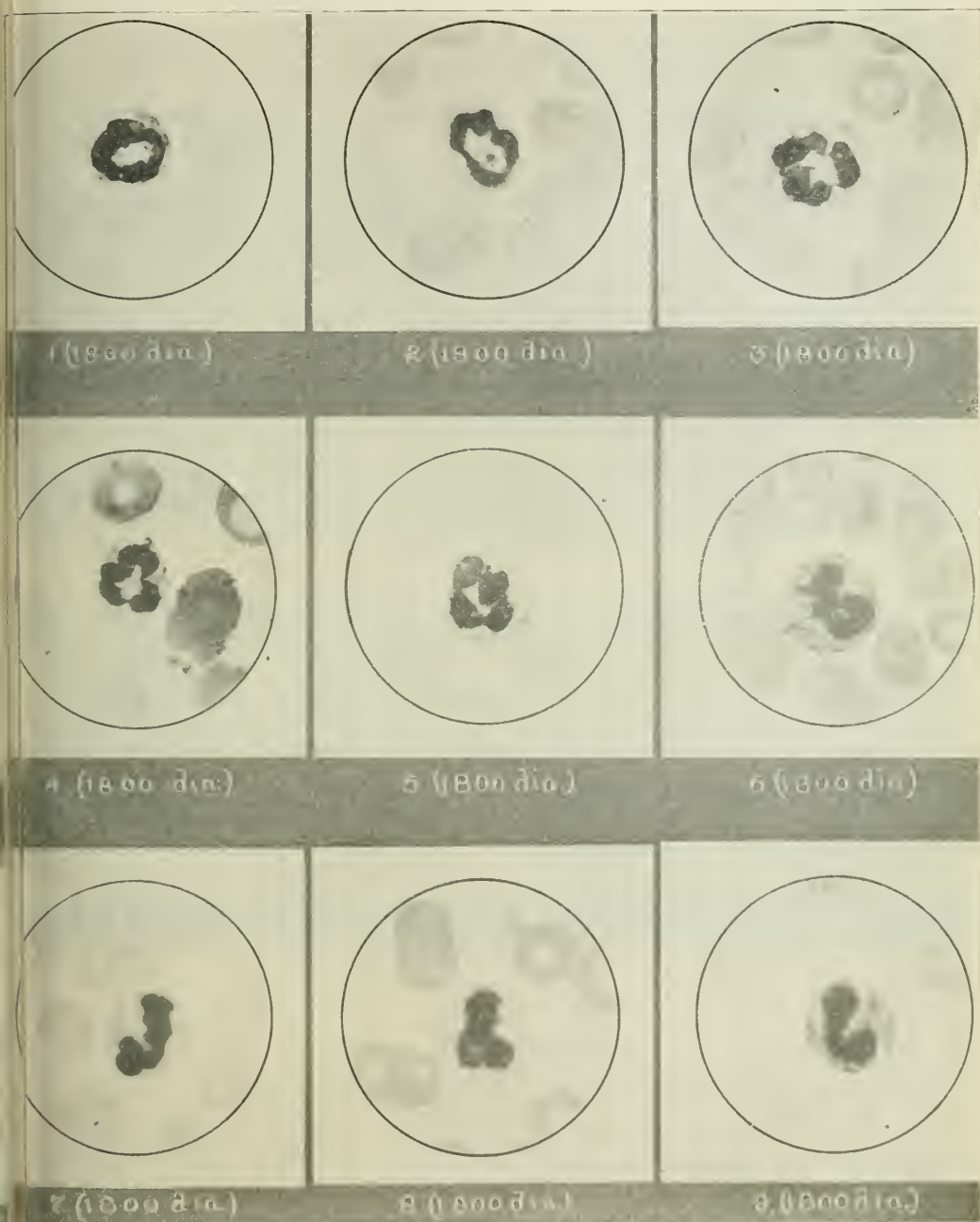


Fig. 3.—Atypical neutrophils in the blood of the writer during exclusive meat diet. (June 27, 1916.)  
Ringforms, 4.5 per cent; Bizarre forms, 9 per cent.

ing. The increase of their numbers in carcinoma, and under certain dietary conditions reported by Gruner and corroborated in the writer's findings, may be important, especially as in the latter instance their increase appeared to accompany the increase of the blood cholesterol. The assumption that their peculiar morphology may be in some way connected with the chemistry of the blood would seem to be supported by Weidenreich's reference to internal causes (conditions of nutrition?) in his classic work on the morphologic evolution of the nucleus in the polymorphonuclear leucocyte. Weidenreich states emphatically that the configuration of the nucleus in the "finely granular" (*feinkörnig*)

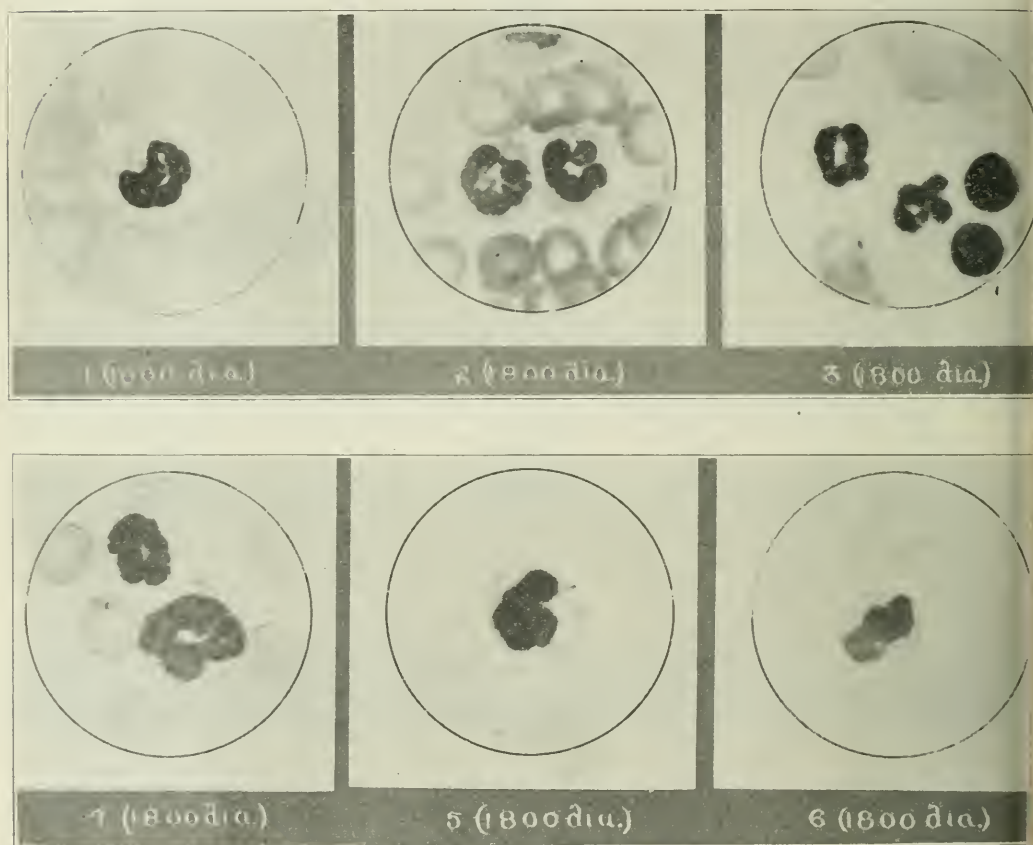


Fig. 3.—Atypical neutrophils in the writer's blood during exclusive meat diet. (June 28, 1916.) Ringforms, 5.6 per cent; Bizarre forms, 5.0 per cent. No. 4, large lymphocyte, lower field.

polymorphonuclears is due to internal causes, as well as to the morphologic differentiation of the nucleus itself. He considers that in perfect specimens the varied shapes of the nuclei are "neither artefacts nor instantaneous pictures of some phase of the motility of the protoplasm" ("... dass die gelappten Kerne der feinkörnigen Leukocyten weder Kunstprodukte noch Augenblicksbilder einer durch die Protoplasmabewegung ständig ummodellierbaren Krenmasse ohne präzisem Formkarakter darstellen.") (p. 239). Only perfect cells that show no trace of crushing or friction have been included in the writer's counts, as may

be seen from the photomicrographs, Figs. 1, 2, 3, 4, 6, and 7. Weidenreich looks upon the more compact nuclei, solid irregular disks, Fig. 2, No. 1, for instance (the solid bars, J and L shapes, Fig. 1, Nos. 7, 8 and 9, may perhaps also come under this heading) as "juvenile forms" (Jugendformen), that will pass through various stages of morphologic differentiation until they present the well-known picture of the fragmented nucleus the component parts of which are united by



A.



B.



C.



D.

Fig. 5.—Scotch oatmeal: Broken oats. Observe that the husks alone have been removed and the slightly hairy capsule is still visible on many of the grains. (Approximately 10 diameters.)

slender threads. The relative position of the fragments may be influenced to a certain extent by the motility of the protoplasm, according to Weidenreich, but this motility plays only a secondary part in the morphology of the nucleus. That any circular arrangement of the nuclear fragments is due to the presence of a "central body" (Centralkörper) he considers "out of the question." True rings and figure-of-eight shapes are found in the blood of rats,<sup>58</sup> (page 226). In human blood the overlapping of the ends of the nuclear thread may produce



similar shapes, but these should be looked upon as "pseudo-rings." This overlapping can be clearly seen in several of the writer's photomicrographs (Fig. 1, Nos. 4, 8, 9; Fig. 3, No. 6; Fig. 4, Nos. 1, 5, 6; Fig. 7, Nos. 1, 2) though in many instances the process of overlapping can hardly be traced (Fig. 1, Nos. 1, 5; Fig. 3, Nos. 1, 2, 3, 4; Fig. 4, No. 1; Fig. 6, No. 2). Two neutrophils

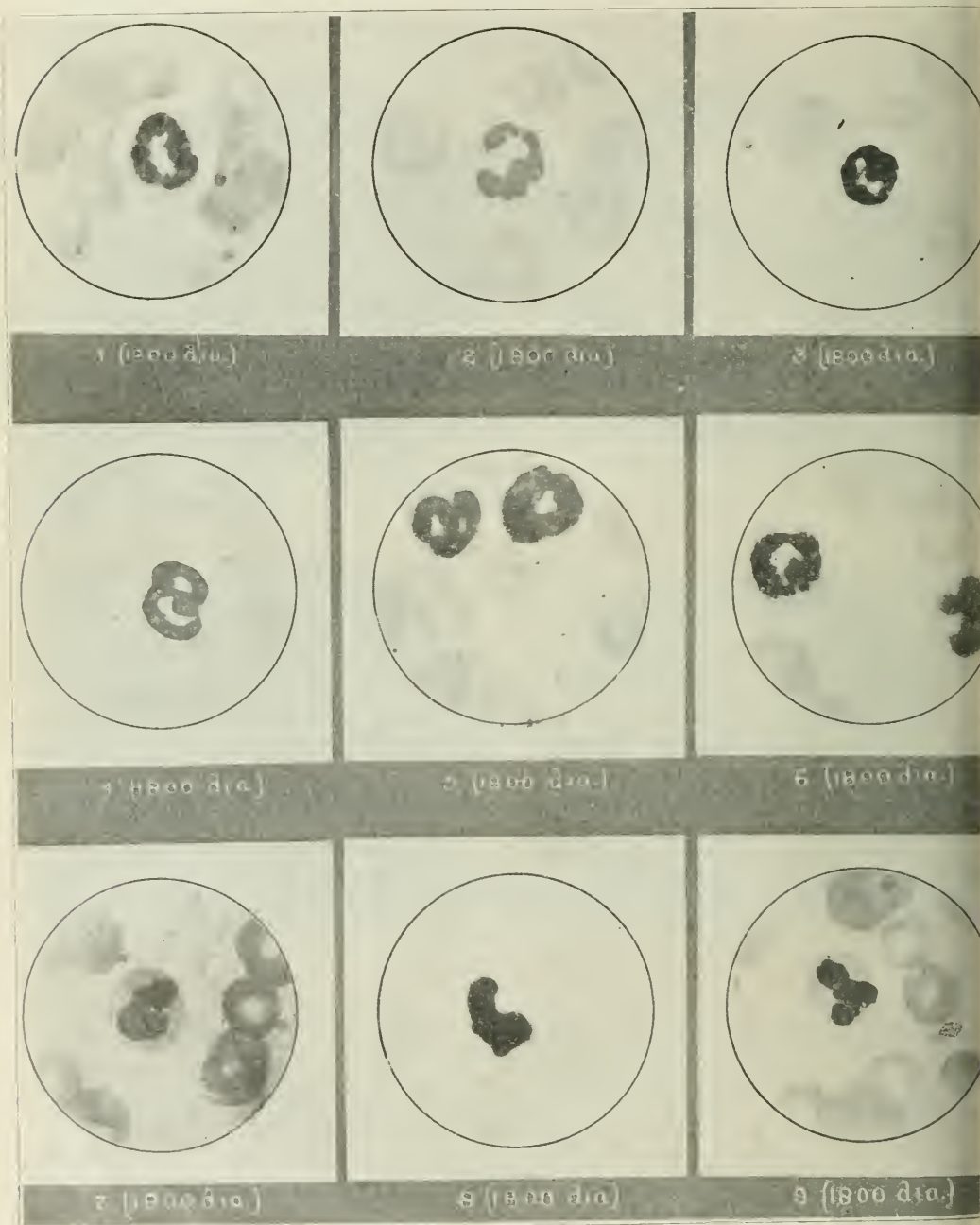


Fig. 6. Atypical neutrophils in the writer's blood during oatmeal diet. (October 31, 1916.) Ringforms, 6 per cent; Bizarre forms 5 per cent. No. 5, large lymphocyte to right.



showing a strong resemblance to a figure eight may be found in Fig. 4, No. 4 and Fig. 7, No. 3. Whatever the cause of the phenomenon may be there seems to be a curious tendency to pseudo-ring formation both in the blood of the carcinoma patient and in that of the writer when the blood cholesterol reaches its maximum. The gradual closing of the ring in the blood of a carcinoma

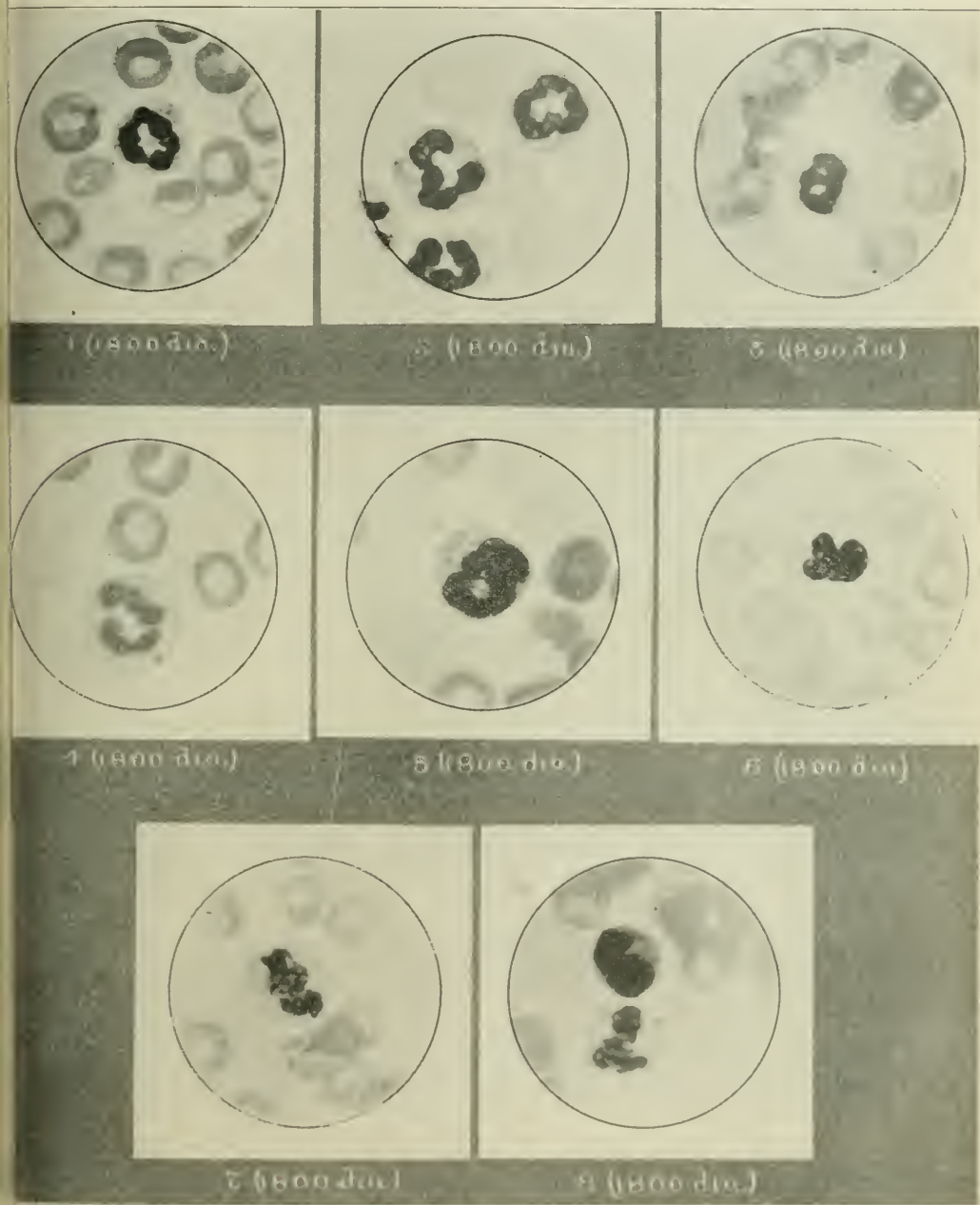


Fig. 7.—Atypical neutrophils in the writer's blood during oatmeal diet. (November 1, 1916.) Ringforms, 6 per cent; Bizarre, 7 per cent. No. 5, large lymphocyte.

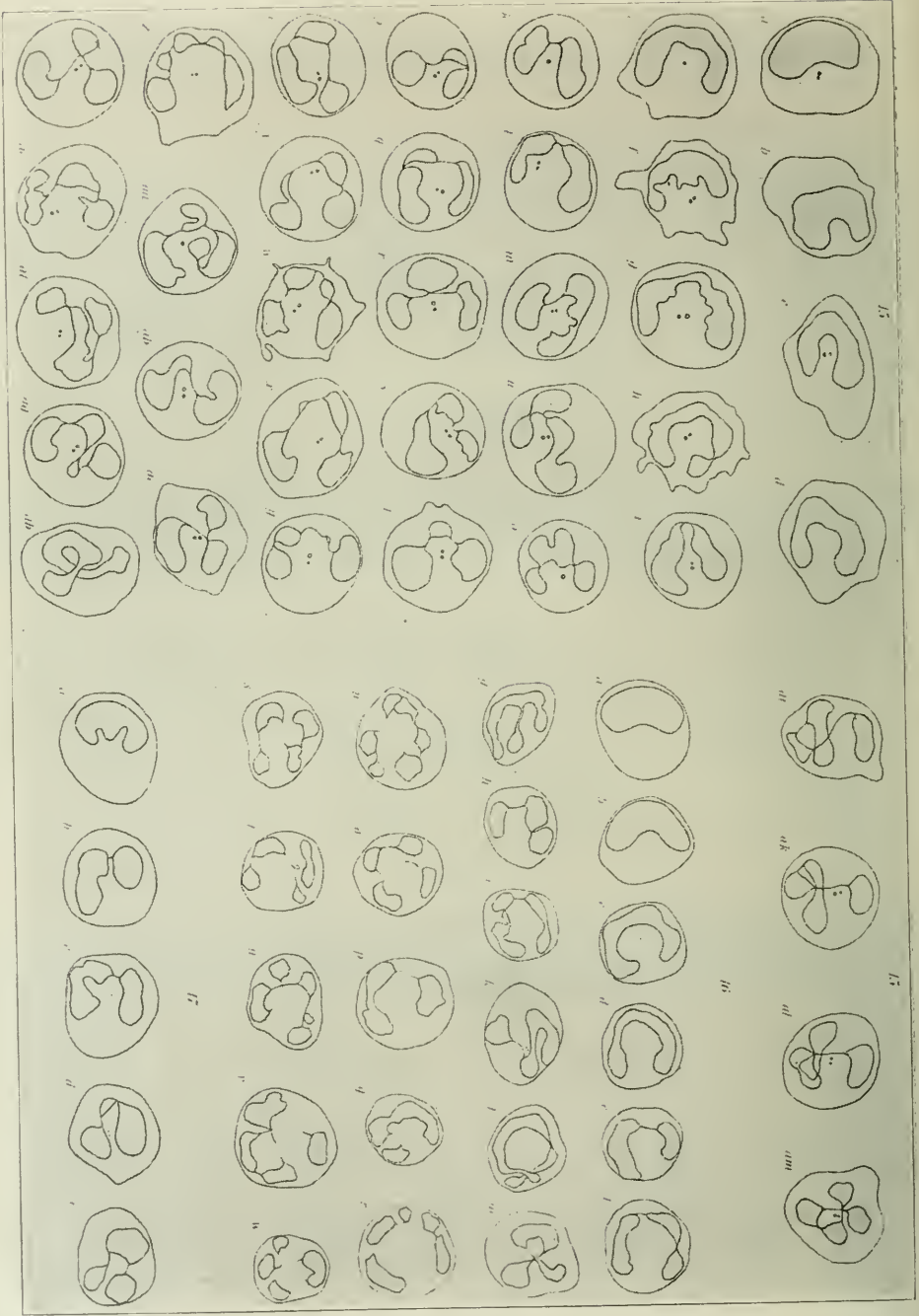


Fig. 8.—Evolution of the nucleus in the neutrophil polymorphonuclear leucocytes. (After Weidenreich.) No. 15, *a* to *am*: Normal human blood. No. 16, *a* to *w*: Leucemic human blood. No. 17, *a* to *e*: Eosinophile leucocytes in leucemic blood. Observe that the only nucleus of the ring type resembling the writer's photomicrographs is in the leucemic blood: No. 16, 1.

patient may be traced in Fig. 1, No. 3. In the blood of the writer, ring-like forms were found even among the large lymphocytes (Fig. 4, No. 4; Fig. 6, No. 5; Fig. 7, No. 5). The morphologic evolution of the nucleus, described by Weidenreich, is illustrated by a reproduction of one of his illustrations (Fig. 8). The fact that the only cell in the diagrams which bears a close resemblance to the writer's photomicrographs, was found by Weidenreich in leukemic blood (Fig. 8, No. 16, 1) may be more than a mere coincidence, since the relation of leucemia, pernicious anemia and carcinoma has been discussed by hematologists as well as pathologists (Pappenheim, Heinrichsdorff, Slye,<sup>50</sup> and Slye, Holmes and Wells<sup>49</sup>). "The leukemic process is to be regarded as a generalized proliferation (Der leukamische Prozess ist als eine generalisierte Wucherung aufzufassend" Grawitz, page 244.) (Figs. 1, 2, 3, 4, 6, 7, and 8.)

Definite conclusions concerning the diagnostic value of these atypical neutrophils are undoubtedly premature, but the fact that their numerical increase is directly proportional to the cholesterol value; i. e., the chemical composition of the blood, both in the diet experiments here reported and in an advanced case of carcinoma, and inversely proportional to the lymphoid count, may be significant; it recalls Weidenreich's suggestion that the study of the atypical leucocytes might not be without practical value and "might open up new perspectives" ("es wäre nicht unmöglich dass sich dadurch neue Gesichtspunkte gewinnen liessen").

That the chemical composition of the blood can be changed by dietetic measures within a few days has been shown repeatedly in these investigations. The blood cholesterol was reduced by one-third in two days by means of Gruner's diet and increased as much in three to four days by the meat and oatmeal experiments. Now if the cytology of the blood and even the morphology of the neutrophil leucocytes can be influenced by the chemical composition of the blood to the extent seen in the experiments here reported, it is hardly to be expected that other cells should be insensitive to this influence. We know that the life of every cell in the body depends on its blood supply. We have seen that the chemical composition of the blood depends to a certain extent, at least, on the chemical composition of the diet. If we consider that dietary conditions may continue unaltered for months and years and that the chemical composition of the blood will affect body cells during this entire period, the influence of the diet on cell growth or cell proliferation can not be discarded as merely hypothetical.

There is every reason to assume that cholesterol plays a part in the formation of new cells. If we admit that cancer, i. e., undue proliferation, almost invariably occurs on the site of old lesions where the normal process of reparative proliferation is already active and further stimulation will be most readily responded to, the significance of the cholesterol content of the blood can not be ignored.

The inheritability of the tendency to tumor formation has been proved repeatedly by Slye<sup>49, 50, 51, 52, 53</sup> in her observations on mice, and the inheritability of specific organs has been shown conclusively by her brilliant report on tumors of the liver<sup>52</sup> in the same animals. In this report sixty-two primary

liver tumors could be traced in the direct descendants of one female mouse with a malignant adenoma of the liver and a sarcoma of the mammary gland.\*

The number of liver tumors and their spontaneous transmission in Slye's mice are equally remarkable as only one tumor of the liver in mice has been reported elsewhere. From the inheritability of specially disposed organs the hereditary transmission of inadequate organs may be logically deduced and if the organs thus transmitted should be among those that take a prominent part in metabolic function (the liver, for instance), faulty metabolism can reasonably be expected in the generations that inherit such organs. The tendency shown by certain types of cancer to appear in various members of human families is not unknown in medical circles,<sup>22, 38, 60</sup> and the assumption that the influence of metabolism on this tendency may be important is supported by Burrow's conclusions from his experiments on the growth of tissue *in vitro*: "If these experiments are substantiated . . . . the problem of cell growth is brought into the domain of chemistry. Thus problems, such as confront us in cancer, are greatly narrowed." It is the writer's conviction that faulty metabolism; i. e., the inability of the organism to metabolize certain kinds of food (meat may be among these), may play a far greater part in the incidence of cancer than has, hitherto, been generally accepted.

#### CONCLUSIONS

A consideration of the work of other observers and of the writer's experiments herein detailed seems to warrant the following conclusions:

1. The influence of the chemical composition of the food on the chemical composition of the blood in increasing or diminishing the amount of cholesterol therein is clearly demonstrated.

2. A diet which increases the blood cholesterol coincidently weakens the lymphoid defense.

3. A diet which reduces the blood cholesterol coincidently increases the lymphoid defense.

4. In persons predisposed to carcinoma an increase of the cholesterol and a weakening of the lymphoid defense such as may occur with the prolonged use of a diet adapted thereto, may perhaps result in the development of carcinoma.

5. Dietetic measures calculated to reduce the blood cholesterol and coincidently increase the lymphoid defense may yet prove to be of value in the treatment of carcinoma.

#### BIBLIOGRAPHY

- <sup>1</sup>Aschoff, L.: Cited by Rothschild,<sup>44</sup> p. 230: Personal communication to Rothschild concerning the presence of gall stones after antifat cures (Entfettungskuren).
- <sup>2</sup>Autenrieth, W., and Funk, A.: Ueber kolorimetrische Bestimmungsmethoden: Die Bestimmung des Gesamtcholesterins im Blut und in Organen, München. med. Wchnschr., 1913, i, 1243-1248.
- <sup>3</sup>Bloor, W. R.: Studies on Blood Fat, II. Fat Absorption and the Blood Lipoids, Jour. Biol. Chem., 1915, xxiii, 317-326.
- <sup>4</sup>Bloor, W. R.: The Determination of Cholesterol in Blood, Jour. Biol. Chem., 1916, xxiv, 227-231.

\*Slye's mice are used for breeding purposes only and the tumors found among them are spontaneous growths in every instance. The incidence of spontaneous tumors in rats has been studied lately by Bullock and Rohdenburg.



- <sup>5</sup>Bloor, W. R., and Knudson, A.: The Separate Determination of Cholesterol and Cholesterol Esters in Small Amounts of Blood, *Jour. Biol. Chem.*, 1916, xxvii, 107-112.
- <sup>6</sup>Bloor, W. R.: The Distribution of Lipoids ("Fat") in Human Blood, *Jour. Biol. Chem.*, 1916, xxv, 577-599.
- <sup>7</sup>Browder, A.: The Effect of Lecithin and Cholesterol Upon the Division Rate of Peramecium, *Univ. California Pub. in Physiol.*, 1915, v, 1-3.
- <sup>8</sup>Bulkley, L. D.: The Relation of Diet to Cancer, *Med. Rec.*, New York, 1914, lxxxvi, 699-702.
- <sup>9</sup>Bullock, F. D., and Rohdenburg, G. L.: Spontaneous Tumors of the Rat, *Jour. Cancer Research*, 1917, ii, 39-60.
- <sup>10</sup>Burrows, M. T.: Some Factors Regulating Growth, *Proc. Am. Assn. Anatomists*, 1916, *Abst. in Anat. Rec.*, 1917, xi, 335-339.
- <sup>11</sup>Chalatow, S. S.: Über das Verhalten der Leber gegenüber den verschiedenen Arten von Speisefett, *Virchows Arch. f. path. Anat.*, 1912, ccvii, 452-476.
- <sup>12</sup>Dezani, S.: Ricerche sulla genesi della cholesterina, I, *Gior. d. r. Accad. di med. di Torino*, 1913, 4s., xix, 149-158.
- <sup>13</sup>Dezani, S.: Ricerche sulla genesi della cholesterina, II, *Arch. farmacol. speriment.*, 1914, xii, 4-12.
- <sup>14</sup>Downey, H.: Personal communication.
- <sup>15</sup>Fodor, A.: Sterine. In *Abderhalden, E.: Biochemisches Handlexikon*, Springer, Berlin, 1914, viii, 474, 476.
- <sup>16</sup>Foote, N. C.: Ueber das Verhalten des Hühnerknochenmarks gegen Immunplasma in den Zellkulturen nach Carrel, *Centralbl. f. allg. Path. u. path. Anat.*, 1912, xxiii, 577-581.
- <sup>17</sup>Foote, N. C.: Über das Wachstum von Knochenmark in vitro, Experimenteller Beiträge zur Entstehung des Fettgewebes, *Beitr. z. path. Anat. u. z. allg. Path.*, 1912, liii, 446-476.
- <sup>18</sup>Friedenwald, J., and Ruhrah, J.: Diet in Health and Disease, W. B. Saunders Co., 1913.
- <sup>19</sup>Gardner, J. A., and Lander, P. E.: The Origin and Destiny of Cholesterol in the Animal Organism. Part XI. The Cholesterol Content of Growing Chickens under Different Diets, *Proc. Roy. Med. and Chir. Soc., London, Series B*, 1913-14, lxxxvii, 229-236, cited by Bloor,<sup>6</sup> page 582.
- <sup>20</sup>Grawitz, E.: *Klinische Pathologie des Blutes*, Leipzig, 1911, Thieme, 204.
- <sup>21</sup>Gruner, O. C.: A Study of the Changes Met with in the Leucocytes in Certain Cases of Malignant Disease, *Brit. Jour. Surg.*, 1916, iii, 506-525.
- <sup>22</sup>Hedinger, E.: Primärer Leberkrebs bei zwei Schwestern, *Centralbl. f. allg. Path. u. path. Anat.*, 1915, xxvi, 385-387.
- <sup>23</sup>Heinrichsdorff, A.: Ueber die Beziehungen der perniziösen Anämie zum Karzinom, *Folia haemat.*, 1912, xiv, *Abst. in Centralbl. f. allg. Path. u. path. Anat.*, 1915, xxvi, 113.
- <sup>24</sup>Hoffman, F. L.: The Mortality from Cancer in the Western Hemisphere, *Jour. Cancer Research*, 1916, i, 21-48.
- <sup>25</sup>Joest, E., and Ernesti, S.: Untersuchungen über spontane Geschwülste, bei Vögeln mit besonderer Berücksichtigung des Haushuhns, *Ztschr. f. Krebsforsch.*, 1915, xv, 1-75.
- <sup>26</sup>Kendall, E. C.: The Isolation in Crystalline Form of the Compound Containing Iodin which Occurs in the Thyroid; Its Chemical Nature and Physiological Activity, *Tr. Assn. Am. Phys.*, 1915.
- <sup>27</sup>Krylow, D. D.: Experimentelle Studien über Nebennierenrinde, *Beitr. z. path. Anat. u. z. allg. Path.*, 1914, lviii, 434-467.
- <sup>28</sup>Luden, G.: Observations on Cholesterol Retention as a Factor in Cell Proliferation, *Jour. Lab. and Clin. Research*, 1916, ix, 662-676.
- <sup>29</sup>Luden, G.: Observations on the Changes in the Cholesterol Content of the Blood of Goats Following Cholesterol Feeding Alone, Roentgen Treatment Alone, and Cholesterol Feeding Combined with Roentgen Treatment and Subsequent Castration, *Jour. Biol. Chem.*, 1916, xxvii, 273-295.
- <sup>30</sup>Levy, R. L., and Rowntree, L. G.: A Study of the Buffer Value of the Blood, *Arch. Int. Med.*, 1916, xvii, 525-539.
- <sup>31</sup>McMeans, J. W.: Tissue Reactions in Experimental Hypercholesterinemia, *Jour. Med. Research*, 1916, xxxiii, 481-491.
- <sup>32</sup>McNee, J. W.: Cholesterin: An Account of Its Relations to Pathology and Physiology, *Quart. Jour. Med.*, 1914, vii, 221-236.
- <sup>33</sup>Müller, F., von, and Bauer, J., von: In their clinical lectures attended by the writer. (Post-Graduate Courses in Clinical Medicine, Munich, 1910-1912.)
- <sup>34</sup>Mueller, J. H.: The Mechanism of Cholesterol Absorption, *Jour. Biol. Chem.*, 1916, xxvii, 463-480.

- <sup>35</sup>Mueller, J. H.: A Comparison of the Results Obtained by the Colorimetric and Gravimetric Determinations of Cholesterol. *Jour. Biol. Chem.*, 1916, xxv, 549-560.
- <sup>36</sup>Murphy, J. B., and Morton, J. J.: The Lymphocyte in Natural and Induced Resistance to Transplanted Cancer, II. Studies in Lymphoid Activity, *Jour. Exper. Med.*, 1915, xxii, 204-211.
- <sup>37</sup>Pappenheim, A.: Perniziöse Anämie und Karzinom in ihren gegenseitigen Beziehungen, *Folia haemat.*, 1912-13, xiv, 329-358.
- <sup>38</sup>Pel, P. K.: Familien-Magenkrebs, *Berl. klin. Wchnschr.*, 1915, lii, 288-289.
- <sup>39</sup>Robertson, T. B., and Burnett, T. C.: The Influence of Lecithin and Cholesterin upon the Growth of Tumors, *Jour. Exper. Med.*, 1913, xvii, 344-352.
- <sup>40</sup>Robertson, T. B.: Experimental Studies on Growth. VIII. The Influence of a Diet Deficient in Fats, and of the Same Diet with Cholesterol Added, upon the Growth of the White Mouse, *Jour. Biol. Chem.*, 1916, xxvii, 393-402.
- <sup>41</sup>Rothschild, M. A.: The Relation of the Liver to the Cholesterin Metabolism, *Proc. New York Path. Soc.*, 1914, n.s., xiv, 229-236.
- <sup>42</sup>Rothschild, M. A.: Zur Physiologie des Cholesterinstoffwechsels. III. Die Beziehungen der Nebenniere zum Cholesterinstoffwechsel, *Beitr. z. path. Anat. u. z. allg. Path.*, 1915, lx, 39-65.
- <sup>43</sup>Rothschild, M. A.: Zur Physiologie des Cholesterinstoffwechsels. IV. Über die Beziehungen der Leber zum Cholesterinstoffwechsel, *Beitr. z. path. Anat. u. z. allg. Path.*, 1915, lx, 1914, lx, 66-75.
- <sup>44</sup>Rothschild, M. A.: Zur Physiologie des Cholesterinstoffwechsels. V. Der Cholesterin-gehalt des Blutes und einiger Organe im Hungerstand, *Beitr. z. path. Anat. u. z. allg. Path.*, 1915, lx, 227-231.
- <sup>45</sup>Rothschild, M. A., and Rosenthal, N.: The Dietetic Management of Hypercholesterinemia in Cases of Cholelithiasis, *Am. Jour. Med. Sc.*, 1916, clii, 394-403.
- <sup>46</sup>Rowntree, L. G.: Personal communication.
- <sup>47</sup>Saltykow, S.: Experimentelle Atherosklerose, *Beitr. z. path. Anat. u. z. allg. Path.*, 1914, lvii, 415-473.
- <sup>48</sup>Simon, C. E.: Personal communication.
- <sup>49</sup>Slye, M., Holmes, H. F., and Wells, H. G.: The Primary Spontaneous Tumors of the Lungs in Mice, Studies on the Incidence and Inheritability of Spontaneous Tumors in Mice; Fourth communication, *Jour. Med. Research*, 1914, xxx, 417-442.
- <sup>50</sup>Slye, M.: The Incidence and Inheritability of Spontaneous Cancer in Mice; Third report, *Jour. Med. Research*, 1915, xxxii, 159-200.
- <sup>51</sup>Slye, M.: The Inheritability of Spontaneous Tumors of Specific Organs and of Specific Types in Mice. Studies in the Incidence and Inheritability of Spontaneous Tumors in Mice; Fifth report, *Jour. Cancer Research*, 1916, i, 479-502.
- <sup>52</sup>Slye, M.: The Inheritability of Spontaneous Tumors of the Liver in Mice. Studies in the Incidence and Inheritability of Spontaneous Tumors in Mice; Seventh report, *Jour. Cancer Research*, 1916, i, 503-522.
- <sup>53</sup>Slye, M., Holmes, H. F., and Wells, H. G.: Primary Spontaneous Sarcoma in Mice; Eighth communication, *Jour. Cancer Research*, 1917, ii, 1-38.
- <sup>54</sup>Soper, W. B.: Zur Physiologie des Cholesterinstoffwechsels. VI. Ueber Beziehungen der Milz zum Cholesterinstoffwechsel, *Beitr. z. path. Anat. u. z. allg. Path.*, 1915, lx, 232-244.
- <sup>55</sup>Stahr, H.: Durch andauernde Haferfütterung erzeugtes Epitheliom der Rattenzunge, *Beitr. z. path. Anat. u. z. allg. Path.*, 1915, lxi, 169-235.
- <sup>56</sup>Sternberg, H.: Die Nebenniere bei physiologischer (Schwangerschaft-) und artifizieller Hypercholesterinämie, *Beitr. z. path. Anat. u. z. allg. Path.*, 1915, lx, 91-123.
- <sup>57</sup>Stevens, R. H.: Blood in Cancer under Roentgen Therapy, *Proc. Am. Roentgen Ray Soc.*, 1916, Abst. in *Jour. Am. Med. Assn.*, 1916, lxxvii, 1549.
- <sup>58</sup>Weidenreich, F.: Beiträge zur Kenntnis der granulierten Leucocyten. V. Fortsetzung der Studien über das Blut und die blutbildenden und zerstörenden organe., *Arch. f. mikros. Anat.*, 1908, lxxii, 209-325.
- <sup>59</sup>Williams, W. R.: The Natural History of Cancer, with Special Reference to its Causation and Prevention, London, 1908, Heineman, 214.
- <sup>60</sup>Wolff, J.: Die Lehre von der Krebskrankheit von den ältesten Zeiten bis zur Gegenwart, Fischer, Jena, 1914, 663. Cited by Hedinger.<sup>22</sup>
- <sup>61</sup>Ziegler, E.: Cited by Naegeli, O.: Blutkrankheiten und Blutdiagnostik; Lehrbuch der morphologische Hämatologie, 2 ed., Leipzig, 1912, Veit, 181.

# ON THE DEVELOPMENT OF A METHOD FOR EARLY DIAGNOSIS OF TUBERCULOSIS BY THE USE OF THE X-RAYED GUINEA PIG\*

BY W. H. ECKFORD, A.B., PITTSBURGH, PA.

THE inoculation method has long been recognized and still stands as the most reliable test at our command for the diagnosis of early or obscure tuberculous lesions. The chief objections to the method are that a positive virus requires a long time to develop in the inoculated animal and that some animals have sufficient natural resistance to overcome a mild infection. For these reasons any means of effectively breaking an animal's resistance so that the length of time is decreased and the "take" made more certain is worthy of a thorough trial.

Murphy and Ellis<sup>1</sup> showed that white mice were remarkably more susceptible to bovine tuberculosis after having been exposed to the x-ray than were normal animals. They offered as an explanation the destruction of the lymphoid tissue which they cited as an important factor in the defensive mechanism against tuberculosis. Morton,<sup>2</sup> assuming that the guinea pig would be affected in a like manner, tried the x-ray on a series of inoculations. He reported that when animals were subjected to a massive dose of x-ray about the time of inoculation, they were so sensitized as to reduce the time of development of recognizable lesions to ten days. He intimated that repeated exposures might be more effective but were unnecessary. He found marked reductions in the leucocyte counts following the exposure.

Haythorn and Lacy,<sup>3</sup> while carrying out some studies in the healing process of tuberculous lesions treated by the x-ray, noted the continued fall in the resistance and the rapid extension of the lesions under frequently repeated large doses of x-ray. Their findings confirmed the decrease in the total number of leucocytes after a large dose of x-ray, but they did not find the decrease in the lymphocytes as described by Murphy and Ellis. They suggested that the well known inhibiting influence of x-ray on the glands of internal secretion might be in a measure responsible for the decreased resistance of the animal.

With the idea of working out a practical routine procedure for sensitizing guinea pigs in the rapid diagnosis of tuberculosis by means of the inoculation method a small group of animals weighing as nearly 260 grams each, as it was possible to get, were inoculated with a uniform dose of tubercle bacilli and given different doses of x-ray at varying intervals. The uniform dose of tubercle bacilli was obtained by adding a few loops of tubercle bacilli from a culture of human bacillus tuberculosis to normal salt and placing the mixture in a vaccine bottle containing glass beads and shaking them for one hour in an electric shaker. An equal amount of 20 per cent India ink† in normal salt was added and one-half cubic centimeter of the suspension injected into both the groin and the peritoneal

\*Reported from the William H. Singer Memorial Research Laboratory, Pittsburgh, Pa.

†The India ink was introduced for the purpose of marking the tubercles so that the results could be used in connection with a separate piece of work.



sac of each of the animals. As the whole group was inoculated from the same suspension, each animal received approximately the same dose of the tubercle bacilli. The dose of x-ray was given with a Coolidge tube. The current was 60,000 volts, spark gap six inches; filter one half millimeter of aluminum; target twelve inches; length of time five minutes. The animals were examined each day and the effects on general condition, time of appearance of nodules, progress of lesions, etc., were noted. Leucocyte and differential counts were made regularly.

Two inoculated animals and a control which received pigment only, were given a single massive dose lasting ten minutes. This was twice the dose given the other animals. All three developed nodules on the fourth day. By the eighth day the nodule in the control had disappeared and those in the inoculated animals had enlarged to the size of a cherry stone. One remained about the same in appearance, the other developed additional nodules, which later united to form a mass one and one-half centimeters in diameter. All three animals retained their sleekness and showed little emaciation at the end of five weeks. They all continued to grow. Leucocyte counts were made on the day before, the day after, and every fifth day after the x-ray exposure. The results were unconvincing. There was an initial leucocytosis due probably to the injection of pigment. This was followed by a decrease to about 50 per cent of the normal count. The differential counts showed the first rise to be due to an increase in polymorphonuclear leucocytes. The later differential counts showed a return to the same relative percentages that were found in the original counts.

A second set of two animals and a control were given the usual dose, the control receiving pigment alone, and were treated on alternate days with a five minute dose of x-ray. The two which received the tubercle bacilli showed nodules in four days, which continued to enlarge. The nodules in one broke down and healed over twice and the animal died on the thirty-third day. At autopsy this animal was found to be greatly emaciated and showed miliary tuberculosis of the peritoneum, lymph nodes, liver, spleen, and lungs. The other animal ran a similar but somewhat longer course. The control showed a nodule at first which had cleared up entirely by the eighth day. The general condition of these animals was bad at all times, they did not grow and while the lesions did not appear any earlier, the extension and development appeared to be more rapid. Leucocyte and differential counts were made on the day following each dose of x-ray. All three animals showed an initial polymorphonuclear leucocytosis followed by a gradual drop to an average of 53 per cent of the original count. The relative number of leucocytes and lymphocytes remained practically the same.

A third set of three was treated on every sixth day and one animal was treated on every third, but the results were so similar to those just cited that no new points can be brought out by giving them in detail. Two animals which received no injections were given x-ray treatments on alternate days, and the leucocytes counted. They showed no initial rise, but the decrease in the cells were exactly similar to those found in the ones which received the inoculations. There was no relative decrease in the lymphocytes. The controls which received neither inoculations nor treatments remained healthy and grew rapidly.

The Singer Laboratory has, since the use of the x-ray for the purpose of



sensitizing animals was begun, the records of fifty-six animals that have been inoculated either with known tuberculous or suspected tuberculous material. In several instances material from a patient has been injected into two animals at the same time, one animal being subjected to x-ray while the other was not. In one such instance both animals showed lesions on the same day, but the rayed nodule outstripped the other in the rapidity of development. In another instance the x-ray was not used until several days after the inoculation, and no nodules had appeared at the time its use was begun. Doses were then given on alternate days and after the second exposure a nodule appeared and developed rapidly.

One animal which had been injected with an unknown fluid for diagnosis was killed and autopsied as soon as a nodule appeared, but neither bacteriologic nor microscopic examination showed the presence of tuberculosis. It later developed that the patient from whom the fluid had been taken had tuberculosis, but the test failed because the animal was killed before the lesions were sufficiently developed for diagnosis. In several other instances growing nodules were removed under local anesthesia and examined, the animal being allowed to live so that the material injected into the peritoneum, if positive, would continue to develop, even if the gland findings were negative. The earliest nodule removed was on the fourth day after inoculation, but neither the bacilli nor characteristic changes were found. One animal, which received a very large dose of known tuberculous material, was autopsied on the tenth day, and tubercle bacilli were present in the smears from the abdominal organs. The glands from the groin showed the earliest recognizable histologic tuberculous rosettes. Most of the animals which showed positive signs were not autopsied under two weeks.

Throughout the work it was obvious that the rapidity of appearance and development of the nodules depended more on the size of the dose of tubercle bacilli than upon the amount of x-ray given.

Based largely upon the above findings the following routine method for using x-rayed guinea pigs in the early diagnosis of tuberculosis, is recommended.

The material must first be prepared for injection. If it is sputum or is of a mucoid nature, it is subjected to fifteen minutes digestion with 4 per cent potassium hydroxide after Petroff's method and is centrifuged and washed before injection. If it is of a fibrinous nature, it is digested in 15 per cent antiformin, the length of time depending upon the resistance of the material to digestion. If it is a clear fluid, it is merely centrifuged and the sediment taken. From one-half to one cubic centimeter of the prepared material is injected into both the groin and the abdomen of the animal, which is then exposed every second day to a five-minute dose of x-ray for at least three exposures. If a nodule has appeared and is still present in two weeks, it is removed under a local anesthetic, smeared, and examined histologically. If positive, it is reported at once; if negative, the animal should be kept for at least six weeks so that in case a few bacilli are present in the cavity they may develop independently from the node in the groin. If the animal is negative in a thoroughly "rayed pig" at the end of six weeks the test may safely be called "negative."

Even in this very small series several points stand out prominently.

1. It is possible by means of the x-ray to increase the susceptibility of guinea

pigs to tuberculosis. Frequently-repeated doses have a more certain effect than a single dose.

2. The destruction of lymphoid cells in itself is insufficient to explain the decreased resistance.

3. The removal of a nodule from the groin for examination can be done in about two weeks and is usually diagnostic. This procedure has the advantage that in case of negative local findings the animal has the chance of developing internal lesions at a slightly later time.

I wish to express my appreciation to Doctors Haythorn, Lacy, and Allison for their interest and suggestions during this work.

#### BIBLIOGRAPHY

<sup>1</sup>Murphy, Jas. B., and Ellis, A. W. M.: Jour. Exper. Med., 1914, xx, 397.

<sup>2</sup>Morton, J. J.: Jour. Exper. Med., 1916, xxiv, 419.

<sup>3</sup>Haythorn, S. R., and Lacy, G. R.: The Effect of X-Ray on Experimental Tubercles Marked with Pigment. Read before the American Association of Pathologists and Bacteriologists, April 7, 1917.

# TUNGSTATES OF ALKALOIDS—AN EXPERIMENTAL PHARMACOLOGIC STUDY\*

BY BERNARD FANTUS, M.S., M.D., CHICAGO, ILL.

## INTRODUCTION

THOUGH tungstates have been used as reagents for alkaloids for many years, the only study of the pharmacologic effects of these alkaloidal combinations, I have been able to find, has been by Carl Nielson,<sup>1</sup> who showed that emetine phosphotungstate is practically nonemetic in dogs, that strychnine phosphotungstate produces convulsions in frogs and in dogs, it being merely somewhat less toxic and slower in action than strychnine sulphate, and that quinine phosphotungstate given to a puppy in doses of 4.65 grains three times daily for ten days, corresponding to a total administration of 117 grains of phosphotungstic acid, produced no evident effects. Inasmuch as the phosphotungstates are much less bitter than the readily soluble salts of the alkaloids, and apparently have less effect upon the stomach, it seemed a subject worthy of further study with a view of the possibility of using these alkaloidal precipitates as an administration form for alkaloids.

There are two questions that must be answered before alkaloidal double tungstates could be seriously recommended for practical use in therapeutics: first, the degree of toxicity or of harmlessness of tungsten and the tungsten acids; and, second, the degree to which the action of the various alkaloids is modified by combination with double tungstates.

Metallic tungsten is considered so harmless that it has been advocated by Richard Krüger<sup>2</sup> as a substitute for bismuth salts in roentgenography of the stomach and intestine. Krüger gave 25 to 80 gm. of colloidal tungsten to human beings without ill effects of any kind.

The most extensive study of the pharmacology of tungsten, at present available, is that of Jacob Bernstein-Kohan,<sup>3</sup> who found that a solution containing less than 5 per cent of sodium tungstate did not produce toxic effects on paramecia, worms or other low organisms. The smallest lethal dose of tungsten, calculated as metal, on subcutaneous administration of sodium tungstate, Bernstein-Kohan found to be for various animal species as follows (Table I):

TABLE I

MINIMUM LETHAL DOSE PER KG. OF ANIMAL, ON SUBCUTANEOUS ADMINISTRATION OF SODIUM TUNGSTATE, CALCULATED AS METAL. (BERNSTEIN-KOHAN.)		
Frog:	About 0.450 gm.	Lived 12 to 13 hours.
Rat:	0.338 "	" 6 to 10 "
Rabbit:	0.044 "	" 2 to 3 "
Cat:	0.111 "	" 12 to 14 "
Dog:	0.078 "	" "
Rooster:	0.250 "	" 20 to 22 "

\*This research was commenced at the Laboratory of Pharmacology of the University of Michigan and completed at the Pharmacologic Laboratory of the University of Illinois.

In marked contradistinction to the toxicity of sodium tungstate on hypodermic administration, Berstein-Kohan obtained negative results on oral administration to rabbits of 0.017 gm. daily for 45 days. He concluded that it is not absorbed or only very sparingly absorbed through the gastrointestinal mucosa.

Kobert's<sup>4</sup> statements regarding our knowledge on tungsten may be summarized as follows: The water soluble salts of the metal, especially tungstic acid, as well as phosphotungstic acid, are as caustic as the soluble iron salts or even as chromic acid. They produce vomiting by local irritation. Noncorrosive tungsten compounds are, like the corresponding iron compounds, absorbed with difficulty from the intestine. But little is known about their toxic action, when they are given by mouth. When introduced subcutaneously into the body, they are deposited, like iron, in the liver, spleen, muscles, kidney, skin, and especially the bones. Elimination occurs chiefly through the mucosa of the alimentary tract with liability to production of gastroenteritis, especially the diphtheritic form of dysentery. It is eliminated to a lesser degree in the urine. Still tungsten may produce a mild degree of nephritis and hemorrhages from the kidney.

Kobert notes that the use of tungsten-containing dyes is prohibited in Berlin on Police Order of Oct. 25, 1878, though neither corrosion nor other damage has been reported from them.

Kunkel<sup>5</sup> also notes that economic, industrial, or experimental intoxication from oral administration of tungsten has not been reported; and that, owing to slight absorption from the intestinal tract, its occurrence is not at all probable.

#### PRESENT STUDY

This work was carried on with phosphotungstic and silicotungstic acids. The latter was used chiefly, because it was found that most of the alkaloidal silicotungstates were less bitter than the phosphotungstates.

Inasmuch as silicotungstic acid does not seem to be readily obtainable on the market, it might be well to describe here the process I employed in the preparation of the substance used in these experiments.

*Preparation of Potassium Silicotungstate.*<sup>6</sup>—To 40 gm.  $\text{Na}_2\text{SiO}_4$  dissolved in 200 ml. water, to make a fairly saturated solution, add 20 ml. conc.  $\text{HNO}_3$  or until the fluid produces a slight reddening of litmus paper. Filter.

To the  $\text{SiO}_2$ , thus produced, was added a solution of 120 gm.  $\text{Na}_2\text{WO}_3$  in 300 ml. of water (to make the total weight of fluid about 8 times that of  $\text{WO}_3$  used). The mixture was next strongly acidified with (75 ml.)  $\text{HC}_2\text{H}_3\text{O}_2$  and heated to boiling.

When the fluid is no longer precipitated within a few moments by  $\text{HCl}$ , permit to cool, filter the feebly acid solution, and add ether and liberal amounts (800 ml. or more) of 33 per cent  $\text{H}_2\text{SO}_4$  until a turbidity is produced on stirring, and three strata are formed on standing. The lower stratum, a molecular combination of ether with silicotungstic acid is, after clearing, separated and decomposed by water. The ether is driven off, and the potassium salts are formed in the following manner:

Into a weighed crucible, place a definite amount, say 2 ml., of the liquid, evaporate to dryness, and then heat to redness. Weigh the residue (which is



chiefly  $\text{SiO}_2$ ,  $12 \text{ WO}_3$ ), and add to 2844 parts of the latter a watery solution of 276 parts of  $\text{K}_2\text{CO}_3$ , and concentrate on the water-bath.

By fractional crystallization, two compounds may be separated from each other. At first crystallizes  $2 \text{ K}_2\text{O}$ ,  $\text{SiO}_2$ ,  $12 \text{ WO}_3$ ,  $18 \text{ H}_2\text{O}$ , in fine hexagonal prisms, which are filtered off as soon as the more soluble orthorhombic crystals of  $2 \text{ K}_2\text{O}$ ,  $\text{SiO}_2$ ,  $13 \text{ WO}_3$ ,  $9 \text{ H}_2\text{O}$  commence to appear. The yield is about 50 per cent of the  $\text{Na}_2 \text{ WO}_4$  used.

### 1. Experiments on the Toxicity of Double Tungstates

*Hypodermic Administration.*—That both phosphotungstic and silicotungstic acids are toxic on subcutaneous administration to guinea pigs, will be seen from Table II which shows the results of injections of 10 per cent solutions of the respective acids.

TABLE II

COMPARATIVE TOXICITY OF PHOSPHOTUNGSTIC AND SILICOTUNGSTIC ACIDS ON GUINEA PIGS

DOSE MG. PER GM.	PHOSPHOTUNGSTIC ACID		SILICOTUNGSTIC ACID	
	NO. OF EXPERIMENTS	RESULTS	NO. OF EXPERIMENTS	RESULTS
0.25	2	Recovered	2	Recovered
0.50	4	All died	6	All died
1.00	1	Died	1	Died

It will be seen that the two acids have the same degree of toxicity, at least within the rather wide limits of this experiment. A dose of  $\frac{1}{2}$  mg. per gram or 0.5 gm. per kg. is uniformly fatal;  $\frac{1}{4}$  mg. per gm. is recovered from. After a dose of 0.5 gm. per kg., the symptoms do not appear for an hour or two. Then the animal develops depression, so that it remains on the side or on the back, when placed in these positions; the breathing becomes labored; and death occurs in from 1 to 3 days. Necropsy shows local irritation and even necrosis at the point of injection.

*Oral Administration.*—In strong contrast to the toxicity of the tungstates on subcutaneous injection is their comparative harmlessness when administered by mouth, as will be seen from the protocols of the following experiments:

PROTOCOL OF PUPPY 1

DATE	DOSE BY STOMACH TUBE	WEIGHT
Aug. 3	0	1,830 gm.
" 8	0.5 gm. Potass. Silicotungstate	1,980 "
" 9	0.5 " " "	2,050 "
" 10	0.5 " " "	1,920 "
" 11	0.5 " " "	1,955 "
" 12	0.5 " " "	1,850 "
" 13	0	
" 14	0.5 gm. Potass. Silicotungstate	2,120 "
" 15	0.5 " " "	2,000 "
" 16	0.5 " " "	2,010 "
" 17	0.5 " " "	2,080 "
" 18	0	2,120 "
" 19	0	2,130 "

Animal seems normal. Urine free from albumin.

The moderate loss of weight produced in Puppy 1, which consumed 4.5 gm. of potassium silicotungstate in nine days, was probably due to temporary digestive impairment, for it was more than made up during the day's interval, on which the drug was not given.

# PROTOCOL OF RABBIT 1

DATE	DAILY FOOD WITH DRUG		(GIVEN DAILY)	URINE QUANT. ML.	WEIGHT (GM.)	REMARKS
	OATS	CARROTS				
Oct. 25	20	100	No medicine	No	925	Ate all
26	"	"	" "	"	930	" "
27	"	"	" "	33	945	" "
28 & 29	40	200	" "	38	945	" "
30	20	100	" "	30	930	" "
31	"	"	" "	21	960	" "
Nov. 1	"	"	" "	20	950	" "
2	"	"	+0.20 Silico- tungstic Acid	10	960	" "
3	"	"	"		970	" "
4 & 5	40	200	+0.40 Silico- tungstic Acid	48	975	" "
6	20	100	+0.20 Silico- tungstic Acid	63	965	" "
7	"	"	"	No		" "
8	"	"	"			" "
9	"	"	"	26	910	" "
10	"	"	"	5	885	Left 30 gm. food
11 & 12	40	200	+0.40 Silico- tung. Acid	38	865	" 65 " "
13	20	100	+0.20 Silico- tung. Acid	45	800	" 60 " "
14	"	"	+0.10 Silico- tung. Acid	23	775	" 30 " "
15	"	"	"	19	770	" 30 " "
16	"	"	"	15	760	" 40 " "
17				5	Died	" 57 " "

The rabbit consumed, in the course of 15 days, 2.3 gm. of silicotungstic acid mixed with its food before it died. Incomplete necropsy showed a small hemorrhage in the cecum and blood stain around the anus.

# PROTOCOL OF CAT 1 (BLACK AND BROWN)

DATE	DAILY FOOD WITH DRUG		(GIVEN DAILY)	WEIGHT (GM.)	REMARKS
	MILK	MEAT			
1916					
Sept. 11 to 14	150	100	None	3,065	
Sept. 14 to 23	"	"	"	3,150	
Sept. 23 to Oct. 2	"	"	"	3,110	
Oct. 2 to 9	"	"	"	3,025	
Oct. 9 to 16	"	"	"	3,105	
Oct. 16 to 26	"	"	"	3,175	

## PROTOCOL OF CAT 1 (BLACK AND BROWN)—CONT'D

DATE	DAILY MILK	FOOD WITH MEAT	DRUG (GIVEN DAILY)	WEIGHT (GM.)	REMARKS
Oct. 26 to Nov. 6	150	100	None	3,025	
Nov. 6 to 9	"	"	"	3,150	
Nov. 9 to 15	"	"	Silicotungstic acid, 2 ml. of 20% solution	3,100	
Nov. 15 to 22	"	"	"	3,165	
Nov. 22 to 30	"	"	"	3,143	Feces fluid for two days.
Nov. 30 to Dec. 4	"	"	"	3,145	
Dec. 4 to 11	"	"	"	3,141	Feces fluid for one day.
Dec. 11 to 18	"	"	"	3,114	Left about $\frac{1}{2}$ food and drug on Dec. 9.
Dec. 18 to 26	"	"	"	3,165	Left about $\frac{1}{2}$ on Dec. 12.
Dec. 26 to Jan. 4/17	"	"	"	2,883	Feces fluid for one day, left dose and milk on Dec. 28 and 29.
1917 Jan. 5 to 13	150	100	Potassium Silicotungstate, 4 ml. of 10% sol.	2,794	Left milk on Jan. 9 and 10.
Jan. 13 to 19	"	"	"	2,644	Left milk on Jan. 14.
Jan. 19 to 30	"	"	"	2,692	Left milk on Jan. 28.
Jan. 30 to Feb. 7	"	"	"	2,635	Left milk on Feb. 2.
Feb. 7 to 15	"	"	"	2,567	Left milk on Feb. 7.
Feb. 15 to Mar. 1	"	"	"	2,015	Left milk on Feb. 15, 17 and 23.
Mar. 1 to 9	"	"	"		Left some of food and drug daily.
Mar. 9 to 28	"	"	None	2,430	Left a little milk occasionally.
Mar. 28 to Apr. 8	"	"	"	3,470	
Apr. 8 to 14	"	"	"	3,357	

Cat No. 1 received 22.40 gm. of silicotungstic acid and 25.20 gm. of potassium silicotungstate, or a total of 47.60 gm. of silicotungstate in the course of 17 weeks. During this period the animal lost about one-third of its weight; but otherwise did not seem to be markedly affected. This loss of weight can hardly be attributed entirely to refusal of food, as this did not occur to a sufficient extent to explain it. Hence there probably occurred either a digestive or a metabolic disturbance, which became only marked during the second half of the feeding period; when even though potassium silicotungstate was given, no change in the downward tendency occurred. That the disturbance was not of a permanent nature, may be seen from the fact that when the drug administration was stopped, the cat rapidly regained and exceeded the weight it had prior to the drug feeding.

PROTOCOL OF CAT No. 2 (GRAY STRIPED)

DATE	DAILY MILK	FOOD WITH MEAT	DRUG (GIVEN DAILY)	WEIGHT (GM.)	REMARKS
1916					
Oct. 20 to 26	150	100	None	2,385	
Oct. 26 to Nov. 6	"	"	"	2,565	
Nov. 6 to 9	"	"	"	2,545	
Nov. 9 to 15	"	"	Silicotungstic acid 2 ml. of 20% sol.	2,620	
Nov. 15 to 20	"	"	"	2,623	Feces fluid for 2 days.
Nov. 20 to 29	"	"	"	2,640	
Nov. 29 to Dec. 4	"	"	"	2,675	
Dec. 4 to 11	"	"	"	2,711	
Dec. 11 to 18	"	"	"	2,931	
Dec. 18 to 26	"	"	"	2,900	
Dec. 26 to Jan. 6, 1917	"	"	"	2,673	Left about $\frac{1}{2}$ of dose and food on Dec. 28 and 29.
Jan. 6 to 13	150	100	Silicotungstic acid, 2 ml. of 20% sol.	2,511	Left all of dose and milk on Jan. 4 and 5.
Jan. 13 to 19	"	"	"	2,289	Left dose and milk on Jan. 9 and 11.
Jan. 19 to 30	"	"	"	2,232	Left dose and milk on Jan. 20 and 28.



## PROTOCOL OF CAT No. 2 (GRAY STRIPED)—CONT'D

DATE	DAILY MILK	FOOD WITH MEAT	DRUG (GIVEN DAILY)	WEIGHT (GM.)	REMARKS
Jan. 30 to Feb. 7	150	100	Silicotungstic acid, 2 ml. of 20% sol.	2,260	Left dose and milk on Feb. 3 and 7.
Feb. 7 to 15	"	"	"	2,300	Left dose and milk on Feb. 9.
Feb. 15 to Mar. 1	"	"	"	2,520	Left food and drug daily. Animal seems depressed.
Mar. 1 to 9	"	"	"		Animal is sick.
Mar. 9 to 28	"	"	None	2,430	Left a little milk occasionally.
Mar. 28 to Apr. 8	"	"	"	2,712	
Apr. 8 to 14	"	"	"	2,916	

## PROTOCOL OF CAT No. 3 (GRAY STRIPED—MALE)

DATE	DAILY MILK	FOOD WITH MEAT	DRUG (GIVEN DAILY)	WEIGHT (GM.)	REMARKS
1916					
Oct. 16 to 26	150	100	None	2,265	
Oct. 26 to Nov. 6	"	"	"	2,260	
Nov. 6 to 9	"	"	"	2,400	
Nov. 9 to 15	"	"	Silicotungstic acid 5 ml. of 20% sol.	2,510	
Nov. 15 to 22	"	"	"	2,416	Liquid bowel movements Nov. 18 to 22.
Nov. 22 to 29	"	"	"	2,398	
Nov. 29 to Dec. 4	"	"	"	2,345	Liquid bowel movements Dec. 2 to 4.
Dec. 4 to 12	"	"	"	2,300	Ate about half Dec. 4 and 12.
Dec. 12 to 18	"	"	"	2,280	Liquid bowel movements, Dec. 14 and 15.
Dec. 18 to 26	"	"	"	2,339	Liquid bowel movements, oc- casionaly.
Dec. 26 to Jan. 3, 1917	"	"	"	2,310	Left most of drug and food after Dec. 29
Jan. 3, 1917	"	"	"		Animal weak and sick. Died 3 P.M.

Cat No. 2 received 47.60 gm. of silicotungstic acid, mixed with its milk, in the course of 17 weeks. During the first seven weeks, the animal gained in weight. During the remainder of the period, the animal left more and more of its food as the time went on, until during the last week it ate very little. At this time the animal seemed quite sick; and it probably would have died if the drug feeding had been continued. To see whether permanent damage was done, the drug feeding was stopped; and complete recovery occurred.

*Necropsy-Report by Dr. D. J. Davis (Univ. of Ill.).*—Extensive hemorrhage in anterior mediastinum and in subpericardial tissue, extending toward visceral pericardium.

Hemorrhage, 2 cm. in diameter, in middle lobe of right lung, involves entire thickness of lung and extends to hilus of the three lobes. Microscopic examination shows the alveoli uniformly filled with blood. The walls are usually distinct, but here and there are broken through; occasionally large regions of hemorrhage appear, containing quite fresh blood. Bronchioles also contain blood. The pleura is normal.

Heart muscle flabby and grayish, small hemorrhage in wall of right auricle, and small subpericardial hemorrhage on left ventricle. Valves, cordæ tendineæ and intima of large vessels, bile stained. Myocardium shows no alterations on microscopic examination.

Liver enlarged, flabby, soft, grayish yellow, greasy on section. Gall bladder moderately distended with yellowish green bile. Microscopically the liver lobules are found to contain large amounts of fat, evenly distributed. At the periphery are deposits of granular biliary pigment. The central veins are normal in appearance. Kupffer's cells contain large quantities of yellow pigment granules.

Spleen slightly pale in color and mottled in places. Pancreas grayish white and soft. Neither spleen nor pancreas reveals microscopic changes worthy of note.

Stomach, negative on gross examination. Microscopically there are found in the mucosa, here and there, small hemorrhages; and, on the surface, rather extensive regions of necrosis in which numerous blood cells appear.

Small intestine, 10 inches above ileo-cecal valve shows submucosal hemorrhages, becoming more frequent and extensive higher up to 10 inches below the stomach. Colon negative.

*Kidneys.*—Capsule strips readily, leaving a smooth surface. Cortex yellowish gray. Medulla quite red. Markings very distinct. Microscopic examination: The cells of the convoluted tubules appear somewhat swollen and the lumen of the tube is frequently filled with granular detritus. Deposits of rather fine yellow granules appear, especially in the cells of the collecting tubules; also here and there throughout the cortex. The glomeruli are normal in appearance, as is also the capsule.

Urine from bladder contains bile, albumin, no sugar, a few casts, and bile-stained leucocytes and epithelial cells. Some spermatozoa.

*Summary.*—Cat No. 3 consumed at least 50 gm. of silicotungstic acid, mixed with milk, in the course of about two months, before death ensued from fatty degeneration of the liver with multiple hemorrhages in various organs and a

moderate degree of nephritis. The necropsy findings remind one somewhat of those of phosphorus poisoning.

### *Summary of the Feeding Experiments*

From the above detailed experiments, it seems evident that silicotungstic acid or potassium silicotungstate may be fed to animals for considerable periods and in fairly large quantities. A dose of 50 gm. of silicotungstic acid, consumed in about 8 weeks, killed cat No. 3, while somewhat less than this quantity (47.60 gm.), given in 17 weeks, was survived by cats Nos. 1 and 2. Hence silicotungstic acid, given by mouth, is not very toxic to cats.

### THE ACTIVITY OF DOUBLE TUNGSTATES OF ALKALOIDS

*Strychnine Phosphotungstate.*—This compound, kindly furnished me by the Abbott Laboratories of Chicago, was administered to two dogs with results shown by the following protocols of the experiments:

*May 29, 1916. Dog 1.*—Weight 4800 gm. Not fed for 24 hours.

8:35 A.M. Strychnine Phosphotungstate 0.038 gm. (= 8 mg. per kg.).

Administered per stomach tube.

8:52 " Violent convulsion.

9:12 " Convulsive starts.

10:30 " Convulsion.

11:30 " Convulsion.

1:15 P.M. Dead.

*June 1, 1916. Dog 2.*—Weight 4500 gm. Just fed.

9:15 A.M. Strychnine phosphotungstate 0.036 gm. (= 8 mg. per kg.).

Administered by stomach tube.

9:45 " Convulsion.

10:15 " Convulsion.

11:40 " Dog standing up. Seems better.

1:30 P.M. Dead.

From these experiments, it will be seen that 8 mg. of strychnine phosphotungstate is fatal to dogs in about 4 hours, convulsions appearing in 16 minutes when the stomach was empty and in 20 minutes when the stomach was full of food. The difference in the condition of the stomach in these two cases evidently made but little difference in the results. Inasmuch as strychnine phosphotungstate represents about 25 per cent of the alkaloid, the dose used corresponds to 2 mg. of strychnine per kg., which is a fatal dose for the dog as will be seen from the following experimental protocols:

*Dog A 1.*—Weight 8500 gm. was given 0.017 gm. strychnine sulphate (= 2 mg. per kg.). Convulsions in 12 minutes, death in 26 minutes.

*Dog A 2.*—Weight 9500 gm. was given 0.019 gm. strychnine sulphate (= 2 mg. per kg.). Convulsion in 10 minutes, death in 11 minutes.

The chief difference, it will be noted, is in the time of the occurrence of death, which was an average of about 18 minutes with strychnine sulphate, and 4 hours with strychnine phosphotungstate. Our results with this compound agree with those of Nielsen.<sup>1</sup>

*Strychnine Silicotungstate* [ $\text{SiO}_2, 12\text{WO}_3, 2\text{H}_2\text{O} \cdot 4\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_2 + 8\text{H}_2\text{O}$   
(Bertrand)]

Inasmuch as Strychnine Silicotungstate is less bitter than the phosphotungstate, it was subjected to tests. It was prepared by mixing boiling solutions of 2 moles of strychnine sulphate  $(\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_2)_2\text{H}_2\text{SO}_4 + 5\text{H}_2\text{O}$  (by weight one part) with 1 mole of potassium silicotungstate— $2\text{K}_2\text{O}, \text{SiO}_2, 12\text{WO}_3, 18\text{H}_2\text{O}$  (by weight two parts). The compound prepared by mixing cold solutions was slightly more bitter than that prepared by the hot process.

That strychnine silicotungstate is highly active, is shown by the following protocols of experiments.

Pup 1, weight 2190 gm., was given 17.5 mg. of strychnine silicotungstate (= 2 mg. of strychnine per kg.) by means of the stomach tube. Convulsions in thirty minutes, death in forty minutes.

Pup 3, weight 1900 gm., was given 15.2 mg. of strychnine silicotungstate (= 2 mg. of strychnine per kg.) by stomach tube. Convulsions in thirty minutes, death in forty minutes.

Cat 1, weight 2670 gm., was given 21.4 mg. strychnine silicotungstate (= 2 mg. of strychnine per kg.) by stomach tube. Convulsions in thirty-five minutes, death within two hours.

Cat 2, weight 800 gm., was given 6 mg. strychnine silicotungstate (= 2 mg. per kg. of strychnine) by stomach tube. Convulsion in twelve minutes, death in twenty minutes.

Cat 3, weight 3850 gm., was given 30.8 mg. strychnine silicotungstate (= 2 mg. per kg. of strychnine) by stomach tube. Convulsions in fifteen minutes, death in twenty-five minutes.

To compare the activity of strychnine silicotungstate with that of strychnine sulphate, the minimal lethal dose of each was determined by experiments upon rabbits, with the result shown in Table III.

TABLE III

COMPARATIVE TOXICITY OF EQUIVALENT DOSES OF STRYCHNINE SULPHATE AND OF STRYCHNINE SILICOTUNGSTATE TO RABBITS

STRYCHNINE SULPHATE				STRYCHNINE SILICOTUNGSTATE			
Dose mg. per kg.	No. of Animals	Results		Dose mg. per kg.	No. of Animals	Results	
		Conv's	Final			Conv's	Final
		In		10	1	5 hours	Recov'd
						In	All
5	6	35 min.	3 died	15	3	4½ hours	Recov'd
						Av.	
				20	8	5½ hours	5 died
						Av.	

It will be seen that the lethal dose of 5 mg. of strychnine sulphate equaled in its effects the dose of 20 mg. of strychnine silicotungstate. Inasmuch as 3 mg. of strychnine silicotungstate represents the same amount of strychnine that is contained in 1 mg. of strychnine sulphate, the relation of toxicity is as 5



to 6.6, a difference that is certainly not great. It is of interest to note the long time that elapsed in the strychnine silicotungstate experiments in the rabbit before convulsions appeared. This is in marked contrast to the comparatively prompt appearance of convulsions in cats and in dogs, in which animals convulsions occur so soon after administration of the dose that it is hard to believe that absorption did not occur from the stomach.

### *Double Tungstates of Emetine*

I prepared emetine silicotungstate, a salmon-colored tasteless powder, by mixing 1 part of emetine hydrochloride with 2.7 parts of potassium silicotungstate. This was compared with emetine phosphotungstate (furnished by the Abbott Laboratories), 3.7 parts of which equal 1 part of emetine hydrochloride. It will be seen that these two preparations are of the same strength.

Experiments upon Mice.—White mice were fed with cakes made of cracker, sugar and water to which a definite amount of the agent to be tested was added. The results are shown in the following table. (Table IV.) The animals that died succumbed within 24 to 48 hours.

TABLE IV

COMPARATIVE TOXICITY OF EQUIVALENT DOSES OF EMETINE PHOSPHOTUNGSTATE AND OF EMETINE SILICOTUNGSTATE FOR WHITE MICE

DOSE PER 20 GM. MOUSE	EMETINE PHOSPHOTUNGSTATE		EMETINE SILICOTUNGSTATE	
	No. of Animals	Results	No. of Animals	Results
2.5 mg.	2	Recovered	3	Recovered
5 mg.	3	"	2	1 died
10 mg.	3	All died	1	died

It will be seen that the emetine double tungstates are both highly and probably approximately equally toxic. Owing to the comparative innocuousness of the tungstates, as shown by our feeding experiments, the toxicity must be ascribed to the emetine.

A few experiments were performed upon cats with the following results:

Cat. 1.—Weight 2700—Aug. 3/16.

10:00 A.M. 1 gm. Emetine Phosphotungstate by stomach tube.  
 11:45 " Emesis.  
 P.M. Slight emesis.  
 Defecation.  
 Recovered.

Cat 2.—Weight 3700—Aug. 5/16.

10:45 A.M. 0.5 gm. Emetine Phosphotungstate by stomach tube.  
 Did not vomit.  
 No other symptoms.

Cat. 2.—Weight 3700—Aug. 14/16.

10:30 A.M. 1 gm. Emetine Silicotungstate by stomach tube.  
 1:30 P.M. Large bowel movements. No emesis.  
 No other symptoms.

These results agree, on the whole, with those of Nielsen who showed that,

while small doses of emetine phosphotungstate did not produce emesis, large doses did; though not nearly to the same extent to which the same dose of emetine hydrochloride would have acted.

### *Quinine Silicotungstate*

That this combination, which may be prepared by precipitating a solution of 1 part of quinine hydrochloride with 2.26 parts of potassium silicotungstate, is not very toxic to a small dog, may be seen from the following experimental protocol:

#### PROTOCOL OF PUP 3.—FEMALE—BLACK AND WHITE

DATE			WEIGHT
Aug. 3			1550
" 7	Quinine Silicotungstate	0.5 gm. by Stomach Tube	1710
" 8	"	"	
" 9	"	"	1590
" 10	"	"	1550
" 11	"	"	1535
" 12	No dose		
" 13	"		
" 14	Quinine Silicotungstate	"	1700
" 15	"	"	1780
" 16	"	"	1780
" 17	"	"	1730
" 18	"	"	1830
" 19	"	"	1920

This animal received 5.5 gm. of quinine silicotungstate in the course of 11 days without the production of unfavorable results, excepting an initial loss of weight, which was rapidly recovered from in spite of continuance of the administration.

That quinine silicotungstate is capable of producing effects, may be seen from the following typical protocol of a mouse experiment:

#### PROTOCOL OF MOUSE 2

DATE	FED CAKE COMPOSED OF	WEIGHT	REMARKS
Aug. 2	Quinine Silicotungstate Cracker Sugar Water	gm. 0.10 2.00 0.50 1.00	15 gm.
" 3	Same dose	16	"
" 4	" "	16	"
" 5	" "	16	"
" 6	" "		Feces greenish gray. Left some.
" 7	" "	14½	"
" 8	" "	15	"
" 9	Dead		Ate little.

This animal consumed, at least, 0.50 gm. of quinine silicotungstate in the course of 7 days before death occurred. That the result was due to the drug is rendered evident by the fact that control animals did well on the food without

the drug and that similar results were obtained with three other mice, whose protocols might be summarized as follows:

*Mouse 1.*—Consumed 0.5 gm. quinine silicotungstate in daily amounts of 0.05 gm. in the course of 12 days, before death occurred.

*Mouse 3.*—Consumed 0.3 gm. quinine silicotungstate in daily amounts of 0.10 gm. in the course of 3 days, before death occurred.

*Mouse 4.*—Consumed 0.6 gm. quinine silicotungstate in daily amounts of 0.10 gm. in the course of 6 days, before death occurred.

It may be of interest here to note that, while mice refused to eat cakes made with quinine sulphate or with potassium silicotungstate, they readily ate cakes made with quinine silicotungstate, evidently because of its comparatively slight bitterness.

#### CONCLUSIONS

1. The comparative tastelessness and slight local effects of phosphotungstates and silicotungstates of alkaloids might make these combinations therapeutically useful.

2. The phosphotungstate and silicotungstate radicals are sufficiently non-toxic to be used for the administration of alkaloids with small dosage, such as strychnine or emetine.

3. Inasmuch as the tungstates, when taken in liberal amounts for a long time, do have a toxic action, it is probably not advisable to use these combinations for the administration of quinine or other alkaloids that would be given in large doses, until further studies of the toxicity of these have been made.

#### BIBLIOGRAPHY

- <sup>1</sup>Nielsen, Carl: The Alkaloidal Phosphotungstates, *Am. Jour. Clin. Med.*, Nov., 1916, 903.
- <sup>2</sup>Krüger, Richard: Kolloidales Wolfram als Ersatz für Wismut bei Roentgenaufnahmen des Magen—und Darmkanals, *München med. Wehnschr.*, 1912, lix, 1910.
- <sup>3</sup>Bernstein-Kohan, Jacob: Über die Wirkungen des Wolfram's, *Arb. d. Pharmk. Inst. zu Dorpat*, Stuttgart, 1890, v, 42-126.
- <sup>4</sup>Kobert, R.: *Lehrbuch der Intoxicationen*, Stuttgart, 1906, 323.
- <sup>5</sup>Kunkel, A. J.: *Handbuch der Toxicologie*, Gustav Fischer, Jena, 1899, 221.
- <sup>6</sup>Gmelin-Kraut, iii, 1, 856.

## THREE CASES WHICH ILLUSTRATE THE CONSEQUENCES OF CORONARY LESIONS\*

BY PAUL G. WOOLLEY, M.D., CINCINNATI, OHIO.

### CASE I.

J. B., Hospital No. 1905, a colored man 39 years old, was admitted to the Cincinnati General Hospital on May 8, 1915, complaining of a sharp pain under the sternum.

According to the Clinical Notes, the *family history* was irrelevant.

*Past History.*—The patient had measles and mumps in infancy. Ten or fifteen years ago he had smallpox; and, twelve years ago, rheumatism. About fifteen years ago he had pneumonia. Seven years before admission he had gonorrhea, and three years before that he had a bubo. He does not smoke or chew, and has used no alcohol since January 1, 1915.

*Present Illness.*—This commenced in July, 1914, when he began to experience dyspnea when working hard or climbing a hill. He had a feeling of fullness in the chest. In February, 1915, he began to have pain in the sternal region. On admission, this pain was described as being of a dull aching sort which, at times, becomes quite sharp. It has forced him to stop work and rest for 10 or 15 minutes, after which he could return to work. For several days the patient has had a cough.

*Present State.*—The patient is a well-developed, and well-nourished colored man. Temperature 100°; pulse 128; respirations 40. General condition fair. The pupils are equal and react to light. The conjunctivæ are muddy, the tongue clean, the teeth and gums "poor." The epitrochlear glands are palpable. The chest is symmetrical. The right lung is resonant anteriorly, but shows a diminished posterior resonance on percussion. On auscultation there are crepitant rales at the left base posteriorly; the breath sounds are bronchial in character. The left border of the heart is normal. The apex is in the nipple line. The rhythm is regular. There is a diastolic murmur at the apex and a systolic in the aortic area. The pulses are small. The liver is enlarged.

*May 9, 1915.*—In the evening the patient fell out of bed and was found sweating profusely, with a complete left sided hemiplegia. The tongue deviated to the left, the left side of the face was smooth and the patient was unable to move the left side.

*May 10, 1915.*—At 9:30 A.M., all evidence of the previous evening's attack had disappeared.

*May 20, 1915.*—The patient complained of pain in the left chest on deep inspiration. There was roughened breathing over the left lower lobe and, on the right, between the scapula and the spinal column. The heart began to show some irregularity.

*May 22, 1915.*—The patient said the pain had shifted to the right lower chest and right hypochondrium. There was slightly prolonged expiration and rough breath sounds over the left base, posteriorly. Over the right lower posteriorly crepitant rales were heard. The patient is spitting flecks of blood, bright red in color.

*June 2, 1915.*—Wassermann reaction strongly positive.

*June 20, 1915.*—At 5:25 A.M., the patient got up, dressed, and went to the sun room. He complained of pain in the cardiac region, became dyspneic and died suddenly before the doctor could be reached.

*Clinical Diagnosis.*—Mitral regurgitation; mitral stenosis; pericarditis; passive congestion of the lungs and liver; syphilitic aortitis; chronic nephritis; coronary sclerosis(?) with thrombosis or embolism (?).

### AUTOPSY PROTOCOL

The body of an extremely well-built, and well-nourished colored adult male, 6 feet long. There was nothing remarkable about the head except that the bridge of the nose was thick and flattened and somewhat larger to the right than to the left of the median line. Across the chest were three hypertrophic linear scars, one opposite the second inter-

\*From the Mary M. Emery Department of Pathology of the University of Cincinnati, and the Pathologic Institute of the Cincinnati General Hospital.



space, another, opposite the third, and the last, opposite the fourth interspace. These were all somewhat longer than the width of the sternum. The lower molars were absent on both sides. The third upper molar was loose in the gum; the second upper molar was carious; the three upper left molars were dead. There was a general pyorrhea alveolaris. There was a perforation of the nasal septum. The peripheral lymph glands were not demonstrably enlarged. The testicles were apparently normal. There was no definite preputial scar. The tibial margins were smooth. There was no postmortem rigidity except in the muscles of the jaw. There was no lividity. The body was still warm.

The left pleural cavity was completely obliterated. The right was completely obliterated antero-laterally and apically. In the upper portion was a collection of a straw-colored serous fluid (500 c.c.). There was a slight increase of pericardial fluid, and over the left ventricle anteriorly, posteriorly, and apically were recent fibrinous adhesions.

There was nothing abnormal about the position or appearances of the intestines. The tongue and posterior pharynx, larynx, esophagus and trachea, were apparently healthy. The tonsils were fibroid and atrophic. The lungs were markedly edematous and somewhat salmon-colored, and showed a mild grade, at least, of passive congestion. Edema was more evident in the left lung. There were two very small subpleural areas of consolidation in the right lung which resembled in form and color, incomplete infarcts. The pleura over them was roughened.

The heart was large and flabby. When it was opened, it appeared that it was markedly dilated, and only moderately hypertrophic. The walls were generally thickened, and not contracted apparently, and were gray and fatty. The valves, with the exception of the aortic, were not deformed, and the aortic only to a slight extent. The right and posterior cusps at their common attachment were adherent and slightly contracted. The papillary muscles were fibroid. The aorta was the seat of a diffuse luetic aortitis with evidence of all stages of the process from fatty degeneration, and patchy opalescent succulent fibrosis, to atheroma and calcification. It was very obviously dilated to perhaps one-half more than its normal size. The mouths of the coronaries were remarkably constricted so that the finest probe could be passed with difficulty into the left and could just be passed easily into the right. The coronaries themselves were not sclerotic. The pericardium, over the left ventricle, was congested, roughened, and covered with a fibrinous exudate, beneath which were numerous small hemorrhages.

The spleen was of moderate size, congested, and somewhat fibrotic. The kidneys were enormously congested, and almost purple in color. The capsules removed with ease, tearing in but a few places. There were a few small retention cysts beneath the capsules and the stellate veins were congested. The cortex was of about normal thickness in the left, while in the right it was increased. The surfaces of both organs were somewhat finely granular. The liver was not especially abnormal. It was congested and apparently fatty. The surface was smooth. The cut surface was distinctly fatty and had an indistinct nutmeg appearance. The gall bladder held a fair quantity of yellowish-green mucoid bile. The ducts were patent. The pancreas and adrenals were not remarkable. The stomach and intestines were congested, and in the ileum the solitary follicles were enlarged, especially near the cecum where they were congested and quite raised above the surface. There were patches of congestion in the large intestine. The appendix was normal.

The prostate and bladder were not abnormal.

*Anatomic Diagnosis.*—Syphilitic aortitis involving the mouths of the coronaries, especially the left; cardiac dilatation; passive congestion and edema of the lungs, and abdominal organs; chronic diffuse nephritis; acute follicular enteritis; acute fibrinous pericarditis; pleural effusions; luetic perforation of the nasal septum; pyorrhea alveolaris.

#### REMARKS

In this case, a luetic process in the aorta affected the tissues just about the coronary openings and gradually led to stenosis of these vessels. This process went on for several months during which time the heart gradually lost its reserve, so that exercise caused myocardial insufficiency, and later dilated so that the valvular orifices dilated and at them murmurs appeared. Finally it happened that while the narrowed coronaries permitted just enough blood to pass to nourish the myocardium while the patient was quiet, not enough was able to pass to nourish

the muscle during even slight exertion; and cardiac failure and death appeared.

In this case there was a general cardiac dilatation which means, of course, a general lack of blood to the myocardium.

#### CASE II.

E. G., Hospital No. B-818, a white man, 77 years old, was admitted to the Cincinnati General Hospital on Feb. 3, 1917, complaining of a "sore leg."

The family history was negative.

He denied venereal disease. He has been married 30 years, and has had 3 children. His wife had no miscarriages.

The present condition dates from the first of the year when both lower legs became, gradually, swollen and red, and walking became difficult and painful.

On admission the patient appeared as an old well-developed and well-nourished man with a temperature of 97, pulse 68, and respirations 24. The tongue was foul and covered with a mucopurulent material. The gums were "poor." The lungs appeared normal. The cardiac rhythm was regular, and the sounds were faint and weak. The abdomen was flat. Upon both lower legs there were large infected ulcerations which were somewhat elevated and very hyperemic. The urine was negative.

The next note made two weeks later says that the condition was improved. A week later the patient was stuporous. There was no further note on the case.

Death occurred on March 14, 1917.

*Clinical Diagnosis.*—Dermatitis.

#### AUTOPSY PROTOCOL (H).

The body was that of a fairly well-nourished, well-built, white man of apparently 80 years. The pupils were equal. Many of the teeth were missing and some of those which remained were decayed. The superficial lymph glands were not appreciably enlarged. The chest was deep and barrel-shaped. Upon the lower two-thirds of the right leg was an area over which the skin was sloughed and upon which there were numerous small ulcers. In a corresponding situation on the left leg was a similarly ulcerated but smaller area of dermatitis.

The body cavities contained no fluid. The mesentery and omentum were well supplied with fat. The appendix was *in situ* and evidently healthy. When the sternum was removed the lungs did not collapse chiefly because of emphysema, for in all other respects these organs were normal, except that in the upper portion of the left lower lobe there was a small infarct, the base of which measured 2 cm. in diameter.

The heart was enlarged to fully twice its normal size. Fully three-fourths of the enlarged organ was occupied by the left ventricle. Nevertheless, all the orifices were dilated. There were no essential valvular changes. The left ventricle was filled in its apical half by a large, partially firm mass of adherent red clot which was attached most firmly to the anterior ventricular wall which was about one-third the normal thickness. Also, externally, the anterior left ventricular surface presented an irregularly shaped softened congested area with rather sharp outlines which represented a myocardial area supplied by the descending branch of the left coronary. Dissection of the coronaries showed that they were generally sclerotic. The left coronary was somewhat narrowed but not exceedingly so, while the descending branch was so sclerotic and so nearly occluded that the finest probe could not be made to pass. The myocardium was generally pale and showed scattered areas of fatty degeneration. The papillary muscles were exceedingly fibrotic. The aorta showed generally distributed fatty patches and white, glistening, firm, fibrotic areas, associated with linear intimal wrinkling. There were also numerous calcified plaques, especially in the abdominal segment.

The liver was small and congested. Section showed the nutmeg appearance of a passive congestion associated with an evident diffuse fibrosis, and some fatty changes. The spleen was not unusual though it was congested. It was of the normal adult size and therefore enlarged.

The kidneys were embedded in large amounts of perirenal fat. The capsules stripped with fair ease leaving somewhat congested, slightly granular, sclerotic surfaces. The venules were prominent. Upon section the parenchyma was decidedly congested. The cortices and medullæ were faint. There was evidently a patchy fatty degeneration of the cortical parenchyma.

The bladder contained a small amount of turbid urine. The prostate was somewhat enlarged. There was nothing remarkable in the gastrointestinal tract or pancreas.

*Anatomic Diagnosis.*—Coronary arteriosclerosis and thrombosis; myocardial infarction; cardiac dilatation; myocardial fibrosis and fatty degeneration; aortic atherosclerosis (luteic?); passive congestion of the abdominal viscera; chronic diffuse nephritis; pulmonary emphysema and infarction; ulcerative dermatitis.



Fig. 1.—Case B. 818. Infarction of the papillary muscle. Myocardial fatty degeneration.

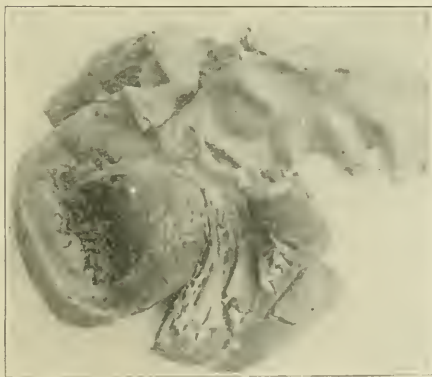


Fig. 2.—Case B. 818. Cardiac hypertrophy and dilatation; ventricular aneurysm (partial); ventricular thrombosis; myocardial fatty degeneration.

#### REMARKS

It seems a pity that the clinical notes in this case are not of some value. Nevertheless the heart illustrates the effect of a localized lack of blood in the myocardium, in contrast with the effects exhibited in Case I.

Here, although there was some general coronary sclerosis, there was little general myocardial disturbance, but in the region supplied by the descending



branch of this vessel, the muscle was badly damaged. The appearances suggest that the sclerotic process had been progressing for some considerable period of time during which the vessel was constricted and also distorted by atheroma. They also suggest that, terminally, a thrombus was formed in the atheromatous portion and that this quite suddenly blocked the vessel and so led to the appearance of the infarct. That there was some time elapsing between the infarction and the older process is suggested by the dilatation of the area of myocardium in which the infarct appeared and by the age of the endocardial portions of the interventricular clot. The lack of blood supply was tending to the production of a cardiac aneurism, a process which was terminated by myocardial death and complete cardiac failure.

### CASE III.

E. W., Hospital No. 6774, a colored woman, 37 years of age, was admitted to the Cincinnati General Hospital on Dec. 21, 1915, complaining of "pain across the chest."

*Family History.*—Negative.

*Past History.*—The patient had been a healthy child. She had the usual diseases of childhood and has had many attacks of sore throat, accompanied by rheumatic pains; but she gave no history of inflammatory rheumatism. Menstruation began at 16 and since then has been accompanied by marked dysmenorrhea. At the age of 32, the menstrual flow became prolonged and excessive, and at 33, a panhysterectomy was done. The patient says a tumor was removed. Since the operation she has been well, though each day she has had to take a cathartic. She has had no children and no miscarriages.

*Present Illness.*—Three weeks before admission, the patient had a gastric upset the cause of which she does not know. This was characterized by vomiting, pain in the chest, headache, and general weakness. It was because of these symptoms that the patient came to the hospital.

*Present State.*—The patient lies quietly in bed and has rapid, shallow respirations, and no movement of the alæ nasi. The pupils are equal and react to light and during accommodation. The gums are slightly pyorrheic. The throat and tonsils are reddened, but upon them there is no exudate. The anterior cervical glands are palpable. The chest is symmetrical, though expansion is slightly greater on the right than on the left. The vocal fremitus is more marked on the right than on the left. On percussion the note over the left apex and infraclavicular space and downward is higher pitched than on the right. Auscultation is entirely negative. The apex beat is neither seen nor felt. The relative cardiac dullness reaches 10.5 cm. to the left in the fifth interspace, and 2.5 cm. to the right in the fourth. There is no abnormal retrosternal dullness. In the apex region a soft, blowing, systolic murmur is heard, well transmitted to the axilla. The second aortic is louder than the second pulmonic. The spleen and liver are not felt. Over the lower right quadrant there is an increased resistance and the patient complains of some tenderness in this region.

*December 22.*—The patient says there is no pain in the chest, though there is marked epigastric tenderness. There is muscle spasm in the upper half of each rectus. The respiratory movements are free. Auscultation over the heart shows a leathery scratchy friction rub heard between the first and second sounds. This is loudest in the fifth interspace between the midsternal and parasternal lines, and is heard as high as the third rib. The second sounds at the base are both accentuated and of about equal intensity. Following the second sound there is a soft systolic murmur heard best in the second and third interspaces on the left. The friction rub is not palpable. The abdominal pain is probably referred diaphragmatic. The apex of the heart is palpable beneath the fifth rib. The relative cardiac dullness is the same as on admission. The cardiohepatic angle is 90°. The pulse is regular, of moderate size, and rather quick. No capillary pulse is seen. The friction rub does not disappear with deep breathing, and is present when the patient holds her breath. (At 5 P.M., the friction rub *had* disappeared and was never heard again.)

*December 23.*—Systolic and diastolic cardiac murmurs are heard. The patient has been nauseated and has vomited frequently.

*December 27.*—Vomiting has disappeared for several days. The pulse rate has slowed.



The size of the heart has remained unchanged and the other phenomena are still present. Blood pressure, systolic 140, diastolic 92.

On admission, the urine was negative. Later it showed finely granular casts. A blood culture was negative on Dec. 31. On admission, the leucocyte count gave 14,200, of which 85 per cent were neutrophils. Hemoglobin, 85 per cent. Later the leucocyte count became normal and remained so. A Wassermann test was positive.

During the hospital period, the patient had several attacks of tachycardia.

The first two days in the hospital, the pulse ran between 120 and 150. The temperature was never above 100.5°. The temperature and pulse became normal and remained so until Jan. 12, when the pulse gradually began to rise to 110. On Jan. 18, the pulse rose to 160 and the temperature to 100.5°. The pulse then gradually returned to normal.

*Clinical Diagnosis.*—Coronary thrombosis (?); syphilis; syphilitic aortitis; aortic insufficiency (relative); healed fibrinous pericarditis.

#### AUTOPSY PROTOCOL

The body was that of a well-nourished, well-built colored woman of about 30 or 35 years of age and 5 feet, 3½ inches tall. There was a very slight edema of the ankles. The teeth were in excellent condition for the most part. The pupils were equal; rigor mortis was absent; postmortem lividity was practically absent. Upon the abdomen, running from the umbilicus to the pubes, was a healed linear scar, 15 centimeters long. Over the chest, extending from nipple to nipple and measuring 9 cm. in width, was an area of pigmentation similar to that produced by the application of a mustard plaster. The finger nails were pale. There were no external signs of traumata. The subcutaneous fat was well developed. In the abdomen was a small amount, a few hundred centimeters, of a rather clear serous fluid. There were numerous old veil-like adhesions between the anterior surface of the liver over both lobes and the parietal peritoneum. One loop of ileum and the omentum were adherent to the anterior abdominal wall just below the umbilicus in the region of an old surgical incision. The liver was enlarged and reached to a point 14 centimeters below the ensiform. The peritoneum covering the intestines, and the parietal peritoneum were smooth and shiny, and showed no evidence of inflammatory changes. The appendix was present, 9 cm. long, and, from its beginning, ran directly upward along the posterior surface of the cecum. The stomach was moderately dilated and had an hour-glass shape. When the sternum was removed, there was a gush of clear serous fluid from each pleural cavity, and the lungs did not collapse. There were a few old adhesions in the upper part (posterior) of the left pleural cavity, none in the right. When the bronchi were cut, it was found that there was a large amount of fluid in them. The mediastinal tissues, especially at the hilum of the lungs, were edematous. There was very little pigmentation, anthracosis, and no congestion. There was crepitation throughout. There was a small amount of clear straw-colored pericardial fluid. The blood was fluid. The omentum was adherent to the posterior part of the wall of the pelvis over the rectum and the sigmoid formed a very sharp long loop just before it entered the pelvis. Behind this loop of sigmoid, the small intestine ran and was adherent to the sigmoid low in the pelvis. The mesenteric lymph glands were moderately hyperplastic. There were old adhesions between the cardiac portion of the stomach and the left lobe of the liver. The uterus, both tubes, and ovaries were missing. The bladder was small and contracted. The stomach was filled with partially digested food. The mucous membrane of the stomach was covered with a thick layer of rather tenacious mucus. There was no evidence of ulceration or scar on the internal surface of the stomach. The duodenum showed no obvious abnormality. The pancreas was not abnormal.

The heart was enlarged, the hypertrophy affecting particularly the left ventricle. The tricuspid orifice admitted the passage of three and a half fingers. The pulmonary orifice was apparently normal. The mitral admitted two fingers; the aortic admitted one and a half fingers. The aortic leaflets were only very slightly thickened at the edges, but the whole aortic region immediately above the valves was tremendously thickened, contracted, sclerotic, and just above the junction of the right and left leaflets, there was an area which resembled true bone, within the aortic wall. This area measured 9 mm. in thickness and there was some evidence, to the eye, of marrow formation in this. The mouth of the right coronary was very much contracted so that only the tip of a fine probe was admitted. The mouth of the other coronary also was contracted, though not to the same degree. The whole arch of the aorta was the seat of a typical syphilitic mesaortitis, with very little calcification but a fair amount of fatty degeneration. The coronaries themselves were not sclerotic. There was no macroscopic evidence of thrombosis or embolism. The walls of

the left ventricle were thickened, firm and contracted. The myocardium showed evidence of fibrosis and also of parenchymatous changes which were particularly well seen in the papillary muscles in which there was evidence of a considerable amount of fatty change. The left papillary muscle appeared to be the seat of an almost complete myomalacia superimposed upon a fibrosis. The other valves of the heart were apparently normal.

The liver, 1280 grams, was flabby, congested and gray. These changes, together with the peripheral adhesions, were the only abnormalities in this organ. The gall bladder was filled with a thin brownish mucoid bile. The ducts were patent. There were no calculi. The spleen was of about normal size. The capsule was slightly and irregularly thickened, and running along the anterior edge, 1 cm. from the edge, was a broad linear scar that had caused a puckering in the organ. On section, the pulp was moist and of good color. The malpighian bodies were not visible. The left kidney was slightly smaller than normal, the capsule was somewhat adherent, though it removed with fair ease and tore the cortex in but a few places. The surface was paler than normal. The cortex was somewhat thinned, as was also the medulla. The line of demarcation between the cortex and medulla was not brilliant. There was moderate congestion of both cortex and medulla with evidence in linear striæ of fibrosis of the pyramids. The right kidney was a little larger, 175 grams, than the left, but the general appearances were the same. The lungs were voluminous, pale, crepitated throughout, and were soggy. The pleural surfaces were smooth except that of the apex of the lower left lobe where there was a mass of tags of old adhesions. There were no adhesions between the lobes. The apices were not scarred. On section, the tissue seemed to be saturated with fluid which dripped from the cut surface. Edema was very well marked. The adrenals showed no obvious changes.

*Anatomic Diagnosis.*—Syphilitic mesaortitis; contraction of the coronary orifices; myocardial fibrosis and degeneration; cardiac hypertrophy and dilatation; slight chronic nephritis; catarrhal gastritis; hysterio-oophorectomy (old).

#### REMARKS

The progress of events in this case may have been somewhat as follows:

As the result of the development of a specific infection which affected, as it usually does, both myocardium and aorta, both became fibrotic and less elastic. As a result of this, particularly of the lack of elasticity, the myocardium underwent hypertrophy. Later, the aortic sclerosis developing about the mouths of the coronaries began to narrow them to such an extent that gradually the supply of blood to the myocardium was reduced to a point when, during exertion, was not sufficient for the needs. Transient myocardial failure resulted, but this passed away with rest. Finally, the coronaries became so diminished in size that even in the absence of exertion not enough blood reached the myocardium and it failed permanently.

#### SUMMARY\*

These cases, taken together, illustrate the effects of gradual cutting down of the blood supply to the myocardium. In two cases the process was diffuse, slow and gradual, and led to general ventricular dilatation. In the other, the process was limited to one coronary and, in particular, to one branch. During a certain period the result was gradual though localized, dilatation tending to the production of a cardiac aneurysm. In the final period the process was abrupt and produced an infarct.

\*Other coronary disease sequels have been commented upon in a former paper on Cardiac Aneurysms, in this journal, 1917, ii, 221.

# LABORATORY METHODS

## THE GERMICIDAL VALUE OF THE COMMON GYNECOLOGIC DOUCHING AGENTS\*

BY J. R. STARK, M.D., CINCINNATI, OHIO

THE prevailing skepticism displayed as to the intrinsic germicidal value of the common gynecologic douching agents has prompted this series of experiments, wherein the solutions were used under conditions approaching as nearly as possible those of the human body.

So far as could be ascertained, the method employed in these investigations is original. The attempt was made to avoid complicated methods, to omit such phases as the Rideal-Walker phenol coefficient, etc. The douches employed at the Cincinnati General Hospital and commonly in use in private practice were used in this instance. To the general physician facts which are stated in simple terms—results which are tabulated or given in practical forms—are of more interest and of more value than more scientifically stated and deeply complicated experiments which are not readily applicable to cases at hand.

### TECHNIC

The solutions used were bichloride of mercury in the strengths of 1:1000, 1:4000, 1:15,000; lysol,  $\frac{1}{2}$  per cent; potassium permanganate, 1:1500; and normal saline as a control. The bacteria employed were stock 24-hour cultures of staphylococcus pyogenes aureus, obtained from a case of furunculosis, B. coli of intestinal origin; and B. subtilis. B. subtilis, a spore-former, was used to determine the action of the germicides on this type of organism.

Suspensions were made of the above fresh 24-hour cultures as in preparing vaccines, and one loopful of this suspension was transferred to a test tube one-third full of sterile normal saline. A loopful of this greatly diluted bacterial suspension was then added to melted plain agar tubes, sufficiently cooled so as not to interfere with bacterial growth. These tubes were rolled in grooves on ice, the agar quickly solidifying and forming a thin, uniform film about the sides and bottom of the test tubes. Owing to the constant amount of agar and the uniform size of the tubes, the films were practically two to three millimeters in thickness and practically uniform. These tubes were placed in the incubator for 24 hours. At the end of this time discrete, superficial, and deep colonies were readily visible. The tubes were then ready for irrigation. These tubes were considered to represent the vagina; the agar representing a smooth mucous membrane covered with bacteria, and the deeper colonies representing collections of organisms in the deeper glands and crypts.

\*From the Mary M. Emery Department of Pathology, University of Cincinnati.



The irrigation apparatus consisted of a four-gallon, distilled water laboratory bottle. From the outlet there extended a Y tube to the branches of which were connected, by short pieces of rubber tubing two long capillary pipettes. A similar outfit was employed which contained sterile normal saline solution. With this, the excess germicide was washed off following irrigation.

The large bottle was filled with the germicidal fluid to be used in each experiment. Two inoculated and incubated roll tubes were then placed in inclined racks. The capillary pipettes were inserted to the bottom of each test tube and the germicidal fluid allowed to run in slowly. The tubes were so placed in the racks that the fluid would rise in the tubes and keep the artificial mucous membrane constantly bathed with germicide. The flow was controlled by screw clamps applied to the rubber tubing so that fifteen minutes were required for the use of one-half pint of the solution. The fluid, after washing over the surface of the agar, rose in the tube, finally trickling out of the mouth. The force of the stream was quite sufficient to wash off many of the superficial colonies, but was not swift enough to loosen the agar. Following this irrigation for fifteen minutes, the tubes were inverted, the germicidal fluid allowed to drip out, and the tube then washed with normal saline solution in order to wash off any excess germicide on the agar. The tubes were again inverted until quite dry, when the cotton plugs were thoroughly flamed and inserted into the tubes (these plugs had been kept in sterile petri dishes during irrigation). The test tubes were again incubated for 24 hours.

In every experiment, ten roll tubes of each of the bacteria were used with each germicidal solution. Each experiment, therefore, represents the action of the germicide in sixty cultured tubes, thirty of the superficial and thirty of the deeper.

Five cubic centimeters of sterile normal saline were added to each of the irrigated tubes—these were gently agitated in order to wash off any superficial growth. In practically all instances the saline contained large floating colonies, or was clouded with bacterial suspension. This saline solution was then transferred to a plain broth medium. In order to obtain the deeper colonies, these were picked out by means of a platinum loop and broken up on the sides of other plain broth tubes. The tubes in each series were numbered from 1 to 10.

This second series was again incubated and examined at the end of 24 hours. All tubes appearing to contain a growth were now subcultured on plain agar slants; the growths were later examined microscopically. All tubes showing no growth were again incubated from one to two weeks. Those tubes which had remained sterile for 24 hours continued to remain sterile for the time of observation. This would serve to demonstrate that in these the action of the germicidal fluid was not retardative in character, but destructive.

In order to be certain that bacteria in the superficial suspensions and deeper bits of agar were transferred, the debris at the bottom of the tubes containing no growth was removed and examined microscopically and in all instances showed bacteria which had evidently been killed.

Another series of experiments was carried out practically in the same manner, with the exception that normal saline was used in the place of a germicide. In this series the bacteria were uninfluenced. This was done in order to prove



that irrigation with germicides was not mechanically eliminative in effect, but truly germicidal.

In the accompanying table the results are arranged so that the figures represent the percentage of the tubes in which the germicide was effective; i. e., bacterial death resulted. The numerator represents results on superficial growth, and the denominator, the deeper effects. It will be seen that *bichloride of mercury* in 1:1000 and 1:4000 strengths destroyed all the bacteria: *B. coli*, the staphylococci, and the spore-former, *B. subtilis*. In the 1:15,000 solution, the spore-former remained viable while the two other types of bacteria were destroyed. *Lysol*,  $\frac{1}{2}$  per cent strength, was about as effective as bichloride of mercury, 1:1500; that is, about 90 per cent efficient. Potassium permanganate in 1:1500 strength was remarkably without effect in any of the types. *Normal saline* did not even act as an effective mechanical eliminant.

TABULATED RESULTS OF EXPERIMENT

SOLUTION	STRENGTH	LOCATION	STAPHYLOCOCCUS	B. COLI	B. SUBTILIS
Bichloride	1:1000	superficial	100	100	100
		deep	100	100	100
Bichloride	1:4000	superficial	100	100	100
		deep	100	100	100
Bichloride	1:15,000	superficial	100	100	0
		deep	100	90	0
Lysol	$\frac{1}{2}\%$	superficial	80	100	0
		deep	100	100	0
Potassium permanganate	1:1500	superficial	0	0	0
		deep	0	0	0
Dakin's solution		superficial	100	80	100
		deep	100	100	100
Normal saline		superficial	0	0	0
		deep	0	0	0

Figures represent the percentage of tubes wherein the germicidal action of the fluids was effective.

Here then, by means of a series of simple experiments, we see the effectiveness of germicidal douching fluids *in vitro*. Of course, the roll tube is an idealized vagina, without folds or pockets for the seclusion of microorganisms, and the two can hardly be compared. Still, if potassium permanganate is ineffective in the roll tube, it surely will have little effect in a vagina. On the other hand, bichloride in 1:1000 and 1:4000 strengths is evidently thoroughly effective in destroying all superficial growth and further penetrating agar two to three millimeters and is here similarly destructive. Bichloride in the higher dilutions (1:15,000) or lysol,  $\frac{1}{2}$  per cent, is apparently just as efficient for the pathogenic types of organisms. Therefore, for those who fear to use bichloride as a douche because of the possibility of bichloride poisoning, the higher dilution would seem to give just as satisfactory results. Lysol,  $\frac{1}{2}$  per cent, is just as effective as the last, and is decidedly less dangerous.

It would have been well to have experimented with the gonococcus in a similar manner, but, owing to the difficulties of its cultivation and its pathogenicity, it was not considered. It is only fair to say, however, that the gonococcus very

probably ranks with the nonspore-forming organisms used in the experiment and should as easily be destroyed.

Because of the present interest in Dakin's solution, this was also added to the series. This solution, from personal experience, has been far too irritating as a douching agent and will not be borne by patients. Still in the short time that it was in contact with the bacteria (15 minutes) it was as efficient as bichloride in 1:1000 strength.

#### CONCLUSIONS

1. Bichloride of Mercury destroyed *B. coli*, *staphylococcus pyogenes aureus*, and the spore-former, *B. subtilis* when employed as a germicide in the preceding experiment in low dilutions (1:1000 and 1:4000) in fifteen minutes.

2. Lysol,  $\frac{1}{2}$  per cent, is as effective a germicide as a 1:15,000 bichloride solution as determined by the same procedure.

3. Potassium permanganate 1:1500 strength, when used in the same manner as lysol and bichloride, is wholly ineffective.

4. The solutions used did not inhibit bacterial growth, but destroyed it wherever effective at all.

5. Washing or irrigating with normal saline will not remove all bacterial growth.

---

### AN EXPERIMENTAL STUDY OF ROOT-FILLED TEETH: PRELIMINARY REPORT

BY M. B. COHEN, M.D., WEST SALEM, OHIO

SINCE the epochal work of Billings and Rosenow, many diseased conditions have been traced to focal infections in the oral cavity; clinicians have learned that the mouth contains something besides the tongue and are intelligently examining the teeth and the tonsils. Until recently medical students and internes were not taught to notice the condition of the gums and teeth or to associate disease in these organs with the patient's bodily ills. They were allowed to gain the impression that these organs served a mechanical purpose only, and that their pathology was of importance only in proportion to the disturbance of local function which it caused. Lately many observers have commented on the remarkable clinical results in arthritis, neuritis, myositis, and other obscure symptom-complexes following proper dental procedures.

Because it is relatively easy to diagnose a case of pyorrhea and to get the cooperation of the patient in carrying out proper methods of treatment, the relation of this disease to many systemic conditions has been established clinically.

There is another type of tooth disease, however, which, while not so spectacular in its local end results, is probably as dangerous to the health of the patient, namely, the periapical infection. Unless accompanied by a fistula, this painless condition was, until recently, undiagnosed or disregarded, as it was a common practice to regard a painless tooth as a healthy one. The routine use of the x-ray has made the diagnosis relatively easy and has instituted a study of this type of infection by many workers. It has been claimed to be possible to completely

sterilize these areas through the root canals, to completely fill the latter, and to eradicate the disease; and roentgenograms have been produced which apparently show a growth of new bone around the tooth apex.

While such results may be possible in the hands of an expert like Callahan, of Cincinnati, it is useless to expect the rank and file of dentistry to do this work, as it requires great patience, accuracy, absolute asepsis, and bacteriologic control of each stage of the operation.

That root canal filling in average hands does not eradicate the disease is shown by the following study of six cases of polyarthrititis.

Sixty-two cultures were made from "locked areas" beyond the apices of eighteen teeth that had been root filled from six months to twenty years previously by sixteen different dentists, some of whom are supposed to be authorities in their profession. The following two methods for obtaining cultures were used: If the culture was made for research purposes only, the gum and alveolar process were isolated with cotton rolls like those used routinely in dentistry, dried, and painted with full strength tincture of iodine. A dental hypodermic syringe, which had been previously boiled and allowed to cool, was fitted with a needle, sterilized in the same way and finally passed through a bunsen flame. A puncture was then made through the gum and alveolar process down to the root tip, and an attempt was made to aspirate. The aspirated material, whether visible or not, was transferred to suitable culture media and taken to the laboratory for study. Care was taken to culture only those teeth which had no pyorrhea pockets surrounding them. If the tooth was to be extracted, an area for two inches around was isolated with cotton rolls and painted with iodine. The region was then infiltrated with novocaine solution which had been boiled and proved to be sterile by bacteriologic checks. The electrocautery was next used around the neck of the tooth and it was extracted through this sterile field with a forceps which had been heated in the flame. The apex was then clipped off into the culture medium by means of a heavy rongeur forceps which had also been flamed.

Growth was obtained without exception from each of the sixty-two cultures on some one of the media used. The organisms usually isolated were those found normally in the mouth. The streptococcus viridans was the predominating one; it occurred in sixty cultures. The staphylococcus family was represented in sixteen, always in combination with the streptococcus, while the colon bacillus was isolated in pure culture once. One culture yielded bacillus acidophilus.

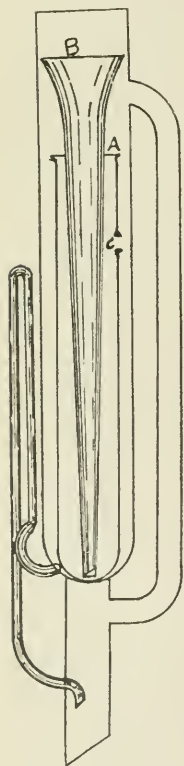
It was not possible to obtain cultures from normal teeth by extraction so that control of the second cultural method described above could not be obtained. The area beyond the apex of one normal tooth was aspirated and cultured for control purposes. This did not yield a growth.

If present day dental technic in average hands does not eradicate this type of mouth infection, it is evident that dead teeth in a patient's mouth are a source of danger. Unless an expert root canal operator is at hand, it is good practice to have such teeth removed. To obtain the best results there must be complete cooperation between the physician and the general dental practitioner that they may decide which teeth should be preserved and which teeth must be removed.

## MODIFICATIONS OF THE SOXHLET EXTRACTOR\*

BY J. W. WEIR, OKLAHOMA CITY, OKLA.

THE Soxhlet extractor requires that the material to be extracted shall be a solid, and, in consequence, liquids or semisolids can be handled only after evaporation to the solid state. The described modification allows of the Soxhlet being used to extract liquids with either lighter or heavier solvents, the only requisite being that the solvent shall be immiscible. When ether or any other solvent floating on the solution being extracted is used, the modification consists of the test tube *A* having a perforation *C* blown in its side at a point higher than the upper level of the Soxhlet siphon. The material to be extracted is placed in this tube *A* and the funnel *B*, made by drawing out a test tube in the blast lamp, is placed inside of *A*. The mouth of this funnel should be large enough to insure the catching of the solvent as it drops from the condenser, but must not fit tightly within the extractor, since vapors must pass around it.



The material being extracted will remain in the bottom of test tube *A*, while the ether or other solvent passes down through *B*, and up through the material being extracted, and overflows through opening *C* into the body of the extractor. When this overflow has reached the crest of the siphon, it will be siphoned off into the lower flask, but as opening *C* is above the level of this siphon the material being extracted is not drawn into the lower flask.

If extraction is being carried on with a solvent which is heavier than the material extracted, the funnel *B* is not used, but is replaced by a second test tube fitting loosely within tube *A* and having a small hole blown in its bottom. This inner test tube must reach well towards the top of the extraction chamber. Test tube *A* is removed from the extractor and filled as far as overflow *C* with the solvent.

The bottom of this test tube is then introduced into *A* far enough that at least one or two inches of solvent rises within it. The sample is then introduced into the inner test tube and the two tubes are placed in the extractor. The solvent drops through the material being extracted in the inner test tube, passes out through the perforated bottom, into the tube *A*, overflowing at *C* and completes the siphoning into the lower flask, as in the first described modification.

For making ether extractions of milk, feces, blood, etc., these modifications of the Soxhlet are decidedly saving of time and in no way affect the accuracy of the extractions.

\*From the Clinical and Research Laboratories of the Wesley Hospital, Oklahoma City, Okla.



# *The Journal of Laboratory and Clinical Medicine*

VOL. III.

DECEMBER, 1917

No. 3

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.

Ann Arbor, Mich.

## ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D.	- - -	ST. LOUIS
HANS ZINSER, M.D.	- - -	NEW YORK
PAUL G. WOOLLEY, M.D.	- - -	CINCINNATI
FREDERICK P. GAY, M.D.	- - -	BERKELEY, CAL.
J. J. R. MACLEOD, M.B.	- - -	CLEVELAND
ROY G. PEARCE, M.D.	- - -	CLEVELAND
ROGER S. MORRIS, M.D.	- - -	CINCINNATI
GERALD B. WEBB, M.D.	- - -	COLORADO SPRINGS
E. E. SOUTHARD, M.D.	- - -	BOSTON

Contents of this Journal Copyright, 1917, by The C. V. Mosby Company—All Rights Reserved  
Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

## EDITORIALS

### *Our Former Teachers in Germany*

MANY medical men in this country have visited Germany for the purpose of continuing their studies. The laboratories and clinics of German universities were quite familiar to American medical men. We know the Anglo-American Societies at Berlin and Vienna and have spent many pleasant evenings at them. There we met many of our colleagues from this side and were introduced to leading German professors who talked to us and considerably offered to form classes in the various medical specialties if so many would attend at so many marks each. We learned much at some of these special clinics and lectures, and our professors were able to convert many dollars into marks. The relations between teacher and students were pleasant and for the most part profitable to both. We learned to admire many of these great teachers and possibly to love a few of them. When the war began our old teachers assumed that their old students would be pro-German. Indeed, they could conceive of nothing else. Probably no one in this country, not of German birth or descent understood the German mind and its attitude toward science and truth better than those of us who had studied in German universities. To us the German professor revealed

himself better and more fully than anyone else could have done. Every lecture and demonstration was an exhibition of his psychology and in this there were many things which no truth-respecting individual could admire. He was generally a learned man in a narrow way, but too often his learning was greatly overshadowed by his arrogance. To him science was "Die deutsche Wissenschaft" and he found frequent opportunity to extol it. He seldom referred to the researches of the men of other nations, and, when he did so, he most authoritatively criticized, minimized, or misstated the facts.

In a course of lectures on the development of abdominal surgery I heard one of the most eminent of German professors say that Bilioth was the first to do an ovariectomy. As I listened I thought of the shades of Ephraim McDowell and his immediate American and English successors, and I wondered whether ignorance or arrogance was the basis of the false statement, and I pitied my German coauditors who evidently accepted it as truth. In this instance I did dare to remonstrate with the learned man at the close of his lecture, but the interview still left me undecided between his ignorance and arrogance. A course of lectures in the history of our knowledge of digestion contained no reference to the work of our Beaumont and Dunglison, although it is true that at the very time that Beaumont was making his classical studies on Alexis St. Martin, the professor of physiology in the University of Berlin was teaching that the stomach is simply a storage and not a digestive organ. I have searched the voluminous German literature on toxins and antitoxins without finding mention of the fundamental researches of Mitchell and Reichert at the University of Pennsylvania or that of Sewall at the University of Michigan. On the other hand, Calmette and other French immunologists, on visiting Ann Arbor, have first of all wished to see the place where Sewall first immunized pigeons to snake venom. The German professor begins the history of tuberculosis with the discovery of the bacillus by Koch, and one would in no way detract from the honor due to the untiring zeal of this great man; but it is a fact that at least a decade before Koch began his work on tuberculosis, Villemin had demonstrated the presence of a virus in the tissue and certain excretions of tuberculous animals, including man. Indeed, he went much further than this and demonstrated the unity of tuberculous infections, a fact up to that time most vigorously denied by Virchow and other German teachers.

We know how the German mind has misunderstood and misinterpreted the teachings of Darwin. In the "survival of the fittest," he has decided that he is the only one "fit." "In the struggle for existence," he has shown himself capable of using the most brutal weapons and to resort to the prostitution of science to the accomplishment of his personal and national desires. His arrogance, so plainly in evidence in his classrooms years ago, has grown into a megalomania which has engulfed the world in a cataclysm and threatens to overthrow the pillars of civilization. The German mind is still in an infantile state, and science in German hands is as dangerous as explosives in a playroom.

American scientists who have studied at German universities are not as a rule pro-German. Individually we respected many of our teachers, but we were not blind to their defects. Because we did not leave the table in rudeness, they

assumed that we enjoyed the food they supplied, and we did. There was some real sustenance in the broth and a few plums in the pudding, enough to make it worth while, since we already had the fundamentals of science and were able for the most part at least to distinguish between the real and the false. In short, our German professors at that time gave us both instruction and amusement. We were not idiots, blind, or deaf, but we did not fully appreciate the pathologic significance of that well-nigh universal German attribute of arrogance and self-conceit. Even at that time it occasionally became boresome and even disgusting, but we did not fully realize its malignant capabilities. It has grown into a great tumor and must be excised if it takes the rest of the world and all time to do it.

Even the tyro in science knows that most of its great discoveries are not of German origin. What of the steam engine, the compass, the telegraph, the telephone, the aeroplane, even the submarine—the list might be indefinitely extended. Science is the exclusive property of no nation; its functions are normally beneficent; its devotees seek the welfare of the race, and not personal or national aggrandizement. Did we have patents on the medicinal uses of quinine, the iodides, the employment of anesthetics and antiseptics? No, we left the patent medicine business to charlatans in this country and German scientists abroad, and both fattened on our good nature and our dollars.

—F. C. V.

---

### *Culture Methods for the Isolation of B. Tuberculosis*

THE isolation and pure cultivation of the tubercle bacillus has from the time of Koch's earliest discoveries been best attained after passage through a guinea pig. Contamination has been too frequent when endeavoring to procure cultures of tubercle bacilli from sputum or tissue in any other manner. A solution of this difficulty was expected in the introduction of antiformin; but this method was soon discarded by bacteriologists. In recent years efforts have been directed to discover a culture medium which would inhibit the growth of extraneous organisms, while permitting the growth of *B. tuberculosis*. Williams and Burdick<sup>1</sup> report the history of this effort.

Von Drigalski and Conradi<sup>2</sup> (1902) observed that crystal violet could inhibit the growth of many bacteria when added to the culture medium; but had no effect on the culture of typhoid or colon bacilli. Churchman<sup>3</sup> (1912) discovered that gentian violet possessed an unusual merit in such selection. By adding gentian violet (0.001%) to his culture medium, Churchman purified a culture of *B. tuberculosis* that had become contaminated with *B. subtilis*. Churchman states that *B. tuberculosis* is "gentian-negative," whereas *B. subtilis* is "gentian-positive," the effect of the gentian being described as "bacteriostatic" rather than bactericidal. Petroff<sup>4</sup> (1915), working with this as a basis, devised a medium composed of meat juice, glycerine, and egg, to which was added minute amounts of gentian violet. Williams and Burdick<sup>1</sup> (1916), among other objections, found that Petroff's medium did not contain enough moisture to prevent rapid drying. They, therefore, devised a new culture medium for the

tubercle bacillus. Williams and Burdick approved the use of sodium (hydroxide) and gentian violet as suggested by Petroff; but employed, as the substance of their medium, egg white, egg yolk, glycerine, meat infusion and agar, a medium on which Besredka had advised the growing of the bacillus for the production of an antigen suitable for complement-fixation tuberculosis tests. Some workers have, we understand, found that the medium of Williams and Burdick also has too rapid a tendency to dry out.

A. S. Griffith,<sup>6</sup> who had so vast an experience in the investigations of the British Royal Commission on Tuberculosis, would seem to have perfected methods of isolating the tubercle bacillus by the aid of antiformin. Some time ago Griffith found that the presence of a small quantity of antiformin in the fluid used for sowing cultures did not interfere with the growth of the tubercle bacillus. He, therefore, merely sowed successive cultures from a mixture of sputum and antiformin after varying intervals. Griffith<sup>7</sup> concludes from his most recent work with animal tissues, as well as with sputum, "that it is more advantageous, especially when dealing with tenacious sputum, to use high percentage of antiformin with short exposure, than low percentage with long exposure."

Griffith (quoted by Cobbett<sup>8</sup>) now makes a 15 per cent addition of antiformin to sputum. "The first sowing should be made one or two minutes after mixture, according to the consistency of the sputum, and three tubes at least ought to be sown within the first five minutes. Thereafter the intervals may be longer—about five minutes, especially if the sputum remains undissolved and mucinous. There is no need to sow tubes after twenty minutes or after the sputum has completely dissolved."

#### BIBLIOGRAPHY

<sup>1</sup>Williams and Burdick: Jour. Bacteriol., July, 1916, i, No. 4.

<sup>2</sup>Von Drigalski and Conradi: Ztschr. f. Hyg. u. Infektionskrankh., 1902, xxxix, 283.

<sup>3</sup>Churchman, J. W.: Jour. Exper. Med., 1912, xvi, 221.

<sup>4</sup>Petroff, S. A.: Jour. Exper. Med., 1915, xxi, 38.

<sup>5</sup>Besredka, A.: Compt. rend. Acad. d. sc., clvi, 1633.

<sup>6</sup>Griffith, A. S.: Brit. Med. Jour., 1914, i, 1171.

<sup>7</sup>Griffith, A. S.: Lancet, London, 1916, i, 723.

<sup>8</sup>Cobbett: The Causes of Tuberculosis, Cambridge, 1917.

—G. B. W.



# *The Journal of Laboratory and Clinical Medicine*

VOL. III.

ST. LOUIS, JANUARY, 1918

No. 4.

## ORIGINAL ARTICLES

---

### ON THE LIBERATION OF EPINEPHRIN FROM THE ADRENAL GLANDS\*

---

WITH DISCUSSION OF SOME OF THE METHODS EMPLOYED IN ITS INVESTIGATION

BY J. M. ROGOFF, M.D., CLEVELAND, OHIO

---

THE organs which furnish internal secretions are attracting the interest of many physiologists, and among this group of important glands the suprarenal bodies have been the subject of considerable investigation, which has resulted in the accumulation of voluminous literature. From time to time various attractive theories have been formulated, some based upon experimental evidence and others being chiefly speculative, in attempts to solve the problems concerning the functions of these glands. The principal result achieved through these theories is the stimulation of the interest of numerous investigators, resulting in the accumulation of data tending to support one or another of the theories, or, on the other hand, establishing evidence which contradicts the fundamental principles upon which they are based. Some of the prominent theories that have been proposed are as follows: that the adrenal glands neutralize toxins circulating in the blood; that the internal secretion of the glands maintains the normal vascular tone; that the glands are interrelated with other ductless glands, directly and indirectly influencing metabolism; that the adrenals have an emergency function, liberating outbursts of epinephrin in times of special stress in response to emotions such as fear, anger, rage, etc., resulting in the mobilization of the defensive forces through the influence of the liberated epinephrin in the blood and its action on the sympathetic nervous system. However, careful consideration of the literature leads only to the conclusion that we are still far from the solution of the problem concerning the function of the adrenals.

---

\*From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, Cleveland, Ohio.

Out of the mass of experimental work on record, there are but few facts that can be accepted as having been definitely established, and these facts at present lend little, or no support, to any of the existing views of the functions of these glands. With the rapid advance being made in the development of physiologic and biochemical methods of investigation it may be hoped that substantial information will be obtained in due time.

Our knowledge of the function of the adrenal glands begins with the observations made by Addison<sup>1</sup> in 1855, that in the disease, which now bears his name, the adrenal glands are the principal seat of pathologic changes. This was followed by experimental investigations made by Brown-Séquard<sup>2</sup> in 1856, who found that these glands are of vital importance, their complete removal in animals resulting in death. The nature of the material manufactured by the adrenals was investigated by Oliver and Schäfer<sup>3</sup> in 1894, and by Szymonowicz<sup>4</sup> in 1895, by injecting into the circulation of animals, extracts of suprarenal glands. They observed that this caused a marked increase in the blood pressure. Later investigations concerning the adrenals were greatly facilitated by the isolation of an active constituent of the glands, which possesses the same blood pressure raising quality as extracts of the gland. In 1897 Abel<sup>5</sup> obtained an active product from the adrenals (epinephrin), and in 1901 Aldrich<sup>6</sup> and Takamine,<sup>7</sup> independently, isolated an active product (adrenalin). This active principle was prepared synthetically by von Fürth<sup>8</sup> in 1898 (suprarenin), and by Dakin<sup>9</sup> in 1905.

The observations of Lewandowsky<sup>10</sup> and of Langley<sup>11</sup> indicated that the active product of the adrenals produced the same effect upon certain structures which receive nerve supply from the sympathetic system, as is caused by electric excitation of their sympathetic innervation. An extensive study of the actions of adrenalin on various structures or organs was made by Elliott<sup>12</sup> and he showed clearly the sympathomimetic action of this substance. This action of adrenalin led to the use of certain structures, which are innervated through the sympathetic system, as biologic test objects whereby the active product of the adrenal glands can be detected and estimated.

A very delicate means of detecting epinephrin in blood is afforded by the use of segments of rabbit's intestine and uterus. Cannon and de la Paz<sup>13</sup> employed for this purpose strips of longitudinal muscle of cat's intestine. Stewart<sup>14</sup> and Hoskins<sup>15</sup> utilized segments of rabbit's intestine and Stewart showed that it is advantageous to use, as an additional test, segments of the rabbit's uterus. The intestine segment, contracting rhythmically in ordinary (venous or arterial) blood, when brought in contact with epinephrin-containing blood responds by inhibition of the tone and contractions of the segment, while the uterus segment indicates the presence of epinephrin by the opposite effect; i. e., by an increase in tone. The importance of corroborating a reaction produced by a specimen of blood upon the intestine with that shown by the uterus, or some other object which reacts in a different manner than the intestine, is evident, since a number of substances other than epinephrin are capable of causing inhibition of the intestine. In fact, minute changes in the concentration of the salts in the fluid which surrounds the segment or alteration of the oxygen supply may cause inhibition. When the inhibition of the segment is due to epinephrin, it can be con-

firmed by the increase in tone produced when the same specimen of blood is applied to the uterus.

Since the proper interpretation of results obtained with the intestine and uterus segments depends greatly upon the manner in which the test is applied, it is not out of place to describe briefly the method of testing blood upon these objects. The apparatus in which the blood is tested consists of a cylinder, in the bottom of which is a hook for the attachment of the segment. A capillary tube enters the side of the cylinder near the bottom. Through this a constant supply of oxygen is kept bubbling through the liquid which surrounds the test-object; the cylinder is kept in a water-bath at a constant temperature of about 38° C. The segment is attached at one end to the hook in the cylinder and at the other end, by a thread, to a lever which records the contractions. The fluid which surrounds the segment is introduced with the aid of a narrow pipette, properly bent and its end drawn out so that it can enter between the segment and the wall of the cylinder, thus permitting the fluid to be introduced at the bottom of the cylinder, so that, as it enters, it can displace the fluid in which the segment is beating. When the test for epinephrin is applied, the segment, contracting rhythmically in Ringer's fluid, is surrounded by ordinary (usually venous) blood which displaces Ringer's solution as it enters the cylinder. The blood to be tested is then introduced through the pipette at the bottom of the cylinder and as it enters it displaces the indifferent blood and the presence of epinephrin is indicated by an inhibition of the tone and contractions of the intestine segment. A doubtful reaction can always be rendered more certain by applying the test to a segment of the rabbit's uterus. When very small quantities of epinephrin are present in a specimen of blood, the test is facilitated by employing the serum, in which the concentration of epinephrin is greater, instead of the whole blood, for it has been shown that in a blood which contains epinephrin, the serum contains the total amount present in the blood.<sup>16</sup> Some investigators remove one fluid from the cylinder before introducing another. This may be a source of error, as exposure of the segment to the air may alter its sensitiveness in succeeding tests. Also, on removal of one liquid, and then replacing another (or this same liquid), the segment will sink, then be floated up with the emptying and refilling of the cylinder, respectively. This will cause the writing lever to record a rise when the segment sinks, and then a fall as the segment is floated up, which may easily be incorrectly interpreted as an inhibition. Displacing one fluid in the cylinder with another by introducing through the pipette with its orifice at the bottom of the cylinder avoids these possible sources of error. The possibility of incomplete displacement by admixture of the fluids is minimized or completely avoided by introducing more displacing fluid than is necessary to just fill the cylinder. Quantitative experiments made in the course of our work with this method have shown that a given specimen of blood yields constant results when applied in successive observations to the same segment. A large number of observations can usually be made on one segment, but it is important to be certain, when comparing the effects produced by various specimens of blood, that the condition of the segment has not altered. A reaction obtained by one specimen early in the series of observations can not be compared with one obtained by a different specimen later on, unless it can be shown by repeated

applications of different specimens of blood that the sensitiveness of the test object is the same.

Since extracts of the adrenal glands, when introduced into the circulation, are capable of influencing the blood pressure, it is desirable to know whether a substance possessing this activity is given off by the glands to the blood which passes through it. To determine this two means are available: (1) the withdrawal of blood through a cannula from the adrenal veins, and testing it on the biologic test objects, or observing its effect when injected into the circulation of another animal; (2) observations in which the liberation of epinephrin is deduced from changes in the blood pressure or other reactions in one and the same animal, without withdrawing the adrenal vein blood.

Evidence of liberation of epinephrin from the adrenals was first obtained by stimulation of the splanchnic nerves. Dreyer<sup>17</sup> and T'sheboksaroff<sup>18</sup> found that blood collected from the adrenal veins of dogs during stimulation of the splanchnic nerves produces a distinct rise in blood pressure when, after defibrination, it is injected into the circulation of another dog.

Employing rabbits' intestine and uterus segments, it was shown by Stewart<sup>19</sup> that blood collected from the adrenal veins during splanchnic stimulation or during massage of the gland, indicated the presence of epinephrin.

Withdrawal of blood, and its defibrination subject it to possible alteration; therefore, it is desirable to correlate the observations already mentioned with results of experiments made on one and the same animal without withdrawing the adrenal vein blood. Meltzer<sup>20</sup> has shown that when the superior cervical ganglion has been excised in a cat, the pupil on the deganglionated side (which becomes constricted) within a few days becomes especially sensitive to adrenalin, dilating widely when adrenalin is introduced into the circulation, while the normal eye remains unchanged. By means of this reaction, Joseph and Meltzer<sup>21</sup> found that stimulation of the peripheral end of the splanchnic nerve in an animal so prepared caused dilatation of the deganglionated pupil. They explained this on the hypothesis of the liberation of epinephrin from the adrenals into the circulation. Stimulation of the splanchnic in the animal in which the reaction is to be elicited renders it necessary to exclude the possibility that the reaction is a nervous phenomenon due directly to the stimulation of the nerves. Asher<sup>22</sup> attempted to determine this point by excising the abdominal viscera, leaving the adrenals intact, then stimulating the splanchnic nerve with the adrenal veins alternately clamped and open. He studied the effects upon the blood pressure and observed that a rise was obtained when the adrenal veins were open, but when they were clamped no rise in blood pressure was caused by splanchnic stimulation. Employing Meltzer's reaction, previously described, Elliott<sup>23</sup> confirmed Meltzer's observation and also found that in cats this reaction is elicited by splanchnic stimulation when the adrenals are intact but could not be obtained after excision of the glands. He further observed that when one adrenal is excised, stimulation of the splanchnic on that side is without effect, while stimulation of the nerve on the side in which the adrenal is intact causes the characteristic effect upon the pupil.

Further evidence that this reaction is obtained through the liberation of epinephrin into the blood coming from the adrenal glands when the splanchnic



nerves are stimulated or the glands massaged has been found in a number of ways by Stewart, Rogoff and Gibson.<sup>24</sup> It was shown that when blood from the adrenals is prevented from reaching the eyeball, as by clamping the adrenal veins or the vena cava (above the orifices of the adrenal veins) during splanchnic stimulation or massage, no reaction is evoked; but when the clamp is released, the reaction occurs after a time interval corresponding to the circulation time necessary for blood to travel from the adrenal to the eyeball. The time required for this reaction to occur can be varied by variations in the rate of the circulation; i. e., slowing the circulation by vagus stimulation or by hemorrhage causes an increase in the time necessary for the reaction to occur, the difference corresponding to the difference in the rate of the circulation. The reaction evoked by massage or splanchnic stimulation under all of the conditions mentioned can be imitated by injecting the proper amount of adrenalin into the circulation at the level of the adrenals, the time necessary before the reaction occurs corresponding with the circulation time as modified by the conditions interposed. The latent period for the secretion of epinephrin is apparently very short, since the time interval necessary to obtain a given reaction is the same with splanchnic stimulation as when a corresponding amount of adrenalin is injected into the circulation at the level of the adrenals.

The evidence of liberation of epinephrin into the circulation from the adrenal glands in response to artificial stimulation of their splanchnic innervation is conclusive, and the next question to consider is whether it is possible to demonstrate liberation of epinephrin from the glands in the absence of artificial stimulation of their nerves, or what may be termed a "spontaneous liberation."

It was found by Tschoboksaroff<sup>18</sup> that adrenal vein blood collected in the absence of splanchnic stimulation caused a smaller rise in blood pressure, when intravenously injected into another dog, than blood collected during stimulation of the nerves, and that after section of the splanchnic nerves the result indicated a distinct diminution in the adrenalin secretion. O'Connor<sup>25</sup> compared on the frog perfusion preparation (Laewen), the constrictor effects of rabbit's adrenal blood collected before and after section of the splanchnics, and observed a greater vasoconstrictor effect to be produced by the blood obtained before the nerve section. He also showed that shed blood develops vasoconstrictor substances. Trendelenburg<sup>26</sup> further observed that these substances are developed in citrate plasma so rapidly (a fraction of a minute) that the perfusion test for epinephrin when present in small quantities can not be entirely reliable.

It is desirable, therefore, to employ a method which eliminates the obvious difficulties entailed in using shed blood and vasoconstrictor reactions for the tests. A convenient method was employed by us,<sup>27</sup> working with cats and dogs. The blood coming from the adrenal veins was collected in a pocket of the vena cava and then released into the circulation, and simultaneous observations made upon the effects produced on the blood pressure of the animal and the eye reactions of Meltzer. The cava pocket is made by tying off the lumbar and renal veins, and all small branches which enter the cava from the liver to the bifurcation of the iliaes; thus a clamp adjusted just below the liver and one just above the iliaes completes a blind pouch into which only the adrenal veins are emptied. Blood collected in such a pocket is released by removing the upper

clamp and the reactions obtained represent those of unaltered adrenal vein blood. By timing the filling of the pocket and also determining the capacity of the pocket, it is possible with this method to determine the rate of blood flow through the adrenals, and by imitating the reaction obtained when the pocket is released by the injection of proper amounts of adrenalin intravenously, it is also possible to estimate the rate of liberation of epinephrin from the adrenals. It has been shown by this method, with the blood pressure and eye reactions, that in the absence of splanchnic stimulation; i. e., spontaneously, a liberation of epinephrin takes place, varying within narrow limits from 0.0008 to 0.0028 mg. per minute in the cat (or from 0.0003 to 0.001 mg. per kilogram of animal). After section of both sympathetics in the thorax near the diaphragm, including both splanchnics, the spontaneous liberation was no longer detected by these reactions, but section of the splanchnics alone in the abdomen did not always entirely abolish the liberation of epinephrin.\*

Electrical stimulation of the peripheral end of the cut nerves during collection of blood in the pocket again elicited the reactions previously obtained. The same result was obtained when the pocket blood was tested upon rabbit's intestine and uterus segments.<sup>28</sup> As the adrenal vein blood, when the segment test is applied, is not diluted with the general blood, as is the case with the test described, these test objects are capable of detecting smaller amounts of epinephrin in the pocket blood than could be detected by the blood pressure or eye reactions. The question may be raised whether this is a normal liberation, or is due to the experimental conditions (trauma, anesthesia, etc.). Although some authors seem to assume that trauma and other experimental conditions will necessarily increase the spontaneous liberation, no experimental proof of this has ever been given. The fact that the output, as experimentally determined, varies within rather narrow limits in different cats, notwithstanding differences in the degree of trauma and the nature and depth of anesthesia, is much in favor of the view that the liberation is a normal phenomenon. We have shown that the anesthetic has nothing to do with it; for (a) the output of epinephrin is within the ordinary limits when anesthesia is produced without the use of a chemical anesthetic, as by a pressure bag inserted intracranially; (b) in animals whose spinal cord has been severed, in the cervical region, some days before the experiment and in which, accordingly, no anesthetic was necessary for testing the adrenal vein blood, the output was also within the usual limits.

It has been established beyond doubt that the adrenal glands continuously secrete a certain normal amount of epinephrin, and that electric excitation of their nerves causes liberation of epinephrin to take place. This leads to the question whether certain physiologic processes are capable of influencing the quantity of epinephrin liberated from these glands.

It has been stated by some writers that the adrenal glands are stimulated to increased secretion of epinephrin by such influences as asphyxia, stimulation of sensory nerves,<sup>29</sup> emotional disturbances<sup>13</sup> and by the introduction into the

\*It is known that all of the secretory fibers to the adrenals do not come from the splanchnic nerves, some of them being given off from the sympathetic lower than the origin of the splanchnics. If there is still a detectable liberation of epinephrin, after section of nerves to the adrenals, some of the nerves going to the glands must have escaped section. In the entire absence of innervation, the adrenals do not liberate epinephrin, and do not seem to regain this power, while the formation is apparently not interfered with after section of the nerves. It must be assumed that after the store, in the glands, has been replenished, the formation stops.

circulation of certain gland extracts.<sup>30</sup> The experimental evidence offered as proof of such an effect upon the adrenal glands is not substantial, as it is based upon investigations made with methods which can not yield reliable results.

For these investigations, blood was collected, with the aid of an aspirator, from the inferior vena cava at the level of the adrenals, by inserting a flexible catheter through an incision in the femoral vein (in cats) so that the opening in the end of the catheter was just anterior to the orifices of the adrenal veins. Specimens of blood were obtained through the catheter before and after causing asphyxia, etc., and were tested for epinephrin on intestine strips or segments. Inhibition of the test object caused by applying the specimen of blood collected after the experimental condition was induced was interpreted as indicating an increase in the amount of epinephrin liberated from the adrenals. It must be pointed out that only the intestine was used as a test object and, as previously stated, this test can be considered reliable only when it is possible to confirm the reaction by some other test object. Indeed, Cannon and Hoskins<sup>29</sup> state that ordinary venous blood collected from an animal during asphyxia causes inhibition of the intestine segment, although they show that this is not due to epinephrin. This fact has been repeatedly observed in the course of our work in this laboratory, and with the aid of the uterus segment we have always been able to eliminate the possibility of such a reaction being due to epinephrin, as it also caused inhibition of the tone of the uterus, while epinephrin has the opposite effect on this test object.

If the blood collected through a catheter from the adrenal level causes an inhibition of the intestine which is due to the presence of epinephrin, this yields no information on the rate of liberation of epinephrin from the adrenals. For this, it is essential to know the rate of the blood flow. A high concentration of epinephrin in the blood may be accompanied by a correspondingly slow blood flow through the adrenal or through the cava, while the liberation of epinephrin from the gland remains constant. It has been shown<sup>31</sup> that within a wide range of variation of blood flow through the adrenals the liberation is approximately constant, and that the concentration of epinephrin in the adrenal vein blood varies inversely with the rate of blood flow through the glands.

It is conceivable that changes in the circulation induced during asphyxia, etc., are capable of sufficiently altering the rate of the circulation in the inferior cava to alter the concentration of epinephrin in the blood which is collected from the cava near the adrenals, but this is not an indication that any change in the rate of liberation of epinephrin from the adrenals has taken place.

Although we have recognized that the catheter method can not yield substantial information, we have made experiments, with this method, on the influence of emotions, asphyxia, and afferent stimulation on the liberation of epinephrin, and have been unable to confirm the results that have been reported.

We have studied the influence of asphyxia and sensory stimulation (of large nerve trunks) upon the liberation of epinephrin from the adrenals,<sup>27</sup> employing the "cava pocket" previously described, with the blood pressure and eye reactions. No effect upon the rate of liberation could be detected by this method. The same result was obtained with the adrenal vein blood, obtained from the cava pocket when tested upon rabbit's intestine and uterus segments.<sup>32, 33</sup> These



observations permitted the study of the rate of blood flow through the adrenals, as well as the concentration of the epinephrin in the adrenal vein blood, and any increase in the concentration could always be accounted for by a corresponding diminution in the rate of blood flow, indicating that no detectable change in the rate of liberation from the glands had taken place during asphyxia or stimulation of the sensory nerves.

Cannon<sup>34</sup> has reported, in a preliminary communication, that he has devised a new method whereby he believes an increased activity of the adrenal is evidenced during asphyxia by a rise in blood pressure which he fails to obtain when the adrenals are excised. The recent observations of Gley and Quinquaud,<sup>35</sup> however, indicate that the rise in blood pressure during asphyxia does not depend upon secretion of epinephrin from the adrenals, for they find no diminution in the rise with asphyxia after ligation of the suprarenal veins. This is against the idea that asphyxia causes any sensible increase in the rate of liberation of epinephrin.

The influence of certain drugs in diminishing the content of epinephrin in the adrenals has led Elliott<sup>23</sup> to interpret the diminished store as due to an increase in the liberation of epinephrin from the gland. He concludes that since morphine, in the cat, produces effects which simulate the general appearance of fright, the resulting diminution of epinephrin store in the adrenals must be due to the increased liberation of epinephrin from the glands in consequence of the emotional excitation or "morphine fright," although he did not directly study the influence of frightening the animals. The effect of morphine in depleting the store of epinephrin from the adrenals has been confirmed by us.<sup>36</sup> But it has been shown that this effect has nothing to do with the emotional state of the cat, since in the dog and rabbit morphine also depletes the epinephrin store from the gland, although the physiologic picture of the animals is the opposite of that in the cat, the narcotic action being manifest. It must be emphasized that diminution of the store of epinephrin in the gland can not be assumed without proof to be the result of increased liberation, as the epinephrin content of the adrenals at any time can only represent the balance between production and liberation. If the rate of production is lessened, the store may be diminished without any change in the output. And contrariwise, if the production is increased, the rate of liberation may also be increased without any diminution in the store. For example, a considerable amount can be caused to be liberated by electric excitation of the splanchnics without noticeable influence on the store of epinephrin in the gland.<sup>24, 36</sup>

There is at present no obvious method by which the influence of emotions upon the rate of liberation of epinephrin from the adrenals can be properly studied. It is not permissible to assume that the symptoms of sympathetic excitation which are seen in a frightened animal must be due to the action of epinephrin liberated in large amounts from the adrenal glands, for the same symptoms can be elicited in animals so prepared that very little or no epinephrin is being given off from the adrenals. By excision of one adrenal and denervation of the opposite gland in the cat it has been shown that the animal can live indefinitely in apparently excellent health, although the operation has reduced the epinephrin from the adrenals to a very small fraction of the normal, and in



many cases to quantities too small for detection with extremely sensitive test objects, indicating practically no liberation whatever.<sup>28</sup> In some of the cats it was found that the liberation of epinephrin from the adrenals was so interfered with by this operation that there could not have been one-thousandth of the normal liberation, if any epinephrin was being given off from the glands. These animals, nevertheless, responded readily to fright and other emotional disturbances with the usual symptoms of sympathetic excitation, in the same manner as normal cats. Certainly, an outburst, through nervous influence, of epinephrin in such quantities as would be necessary to produce these symptoms would be impossible in these animals.

The conception of an emergency function of the adrenal gland lacks substantial evidence. It involves essentially the occurrence of sudden outbursts of epinephrin far above the normal amounts liberated. It has not been shown that the liberation of such amounts of epinephrin can be induced. Even if it could be proved that during such emergency conditions the adrenals responded with augmented liberation, the suggestion that this has a bearing upon the emergency mobilization of sugar in the body, as evidenced by hyperglycemia<sup>37</sup> is not tenable, since it has been shown that cats so prepared that the liberation of epinephrin from the adrenals is reduced to quantities too minute for detection are still capable of responding to the usual procedures (asphyxia, etherization, etc.) employed in producing experimental hyperglycemia.<sup>38</sup> The normal blood sugar in these animals was the same as that found in unoperated animals. This is against the idea of the existence of a hypoglycemia due to adrenal deficiency (said to exist after adrenalectomy, in which the animal is moribund). Further evidence against the view that these hyperglycemias are associated with an increased liberation of epinephrin from the adrenals, or that they are similar to the hyperglycemia produced by injection of adrenalin, is the well-known fact that subcutaneous injection of adrenalin causes hyperglycemia more readily than intravenous introduction.

Animals, with practically no epinephrin being given off from their adrenals, as previously stated, are capable of responding to fright, rage, fear, etc., in the same manner as normal animals. They seem to be as capable of muscular exertion and as combative as the normal animals. The fact that such cats live indefinitely in apparently good nutrient condition suggests that epinephrin is probably not indispensable to life and health, unless the retroperitoneal and other chromaffin tissue in the body, which has been shown to contain epinephrin,<sup>39</sup> can compensate for the loss of epinephrin secretion from the adrenals. It has not been shown, however, that such chromaffin tissue liberates epinephrin. It is possible that some other substance, at present unknown, the liberation of which is under nervous influence, may be found in the secretion of the adrenals, the lack of which is responsible for loss of life when the adrenals are extirpated.

A number of writers have attempted to show that the secretion of epinephrin from the adrenals is influenced by shock. Some of the conclusions offered are based upon histologic appearance of the glands, indicating a loss of chromaffin substance. This can not be taken as evidence of an increase in the liberation of epinephrin, for the same reason discussed previously in relation to conclusions based upon a decrease of epinephrin store in the glands; nor is it likely that his-

tologic examination of the glands could yield definite quantitative information.

An investigation in which adrenal vein blood was collected before and after induction of shock and the bloods tested upon intestine segments was reported by Bedford.<sup>40</sup> He concludes that the rate of liberation of epinephrin from the adrenals in dogs is augmented by shock produced in several ways. The tracings accompanying the paper do not indicate in what manner the test objects were subjected to the action of the bloods. No satisfactory estimations were made to determine quantitatively the amount of epinephrin in the adrenal vein blood collected before and after shock was induced. On careful consideration of the results reported, the only definite information that can be gathered is that if the inhibitions of the intestine were due to epinephrin in the bloods tested, the specimens collected after the induction of shock had a higher concentration of epinephrin than the specimen collected before shock was induced. This increased concentration seems to correspond closely with a fall in the blood pressure. As already pointed out, it is to be expected that in a condition of low blood pressure, when the rate of blood flow through the adrenals is markedly slowed, there will be a relative increase in the concentration of epinephrin in the adrenal blood, although the rate of liberation may be unaltered.

In view of the fact that the adrenal glands secrete a substance so potent as epinephrin, and since it has been shown that the liberation of this secretion into the circulation is sustained through the influence of nerves which are in connection with a definite center in the spinal cord,<sup>41</sup> it would seem not unlikely that certain experimental procedures, such as asphyxia, stimulation of sensory nerves, etc., might be capable of influencing the liberation of epinephrin, reflexly, through this center. This must not be assumed as a fact, however, until it is proved.

Premature attempts on the part of clinical men to apply what is at present known of the physiology of the adrenals, at least when risk to the patient is involved, are to be deprecated. Certainly, the present state of our knowledge of the subject does not warrant its clinical application to the extent of attempting adrenalectomy as a measure of relief in conditions of arterial hypertonus associated with Bright's disease, on the assumption that this condition is associated with hyperadrenalinemia, as has been done.

#### BIBLIOGRAPHY

- <sup>1</sup>Addison, T.: On the Constitutional and Local Effect of Disease of the Suprarenal Bodies, London, 1855.
- <sup>2</sup>Brown-Séquard: Recherches expérimentales sur la physiologie et la pathologie des capsules surrénales, *Compt. rend. Acad. d. sc.*, 1856, xliii, 422.
- <sup>3</sup>Oliver and Schäfer: The Physiological Effects of Extracts of the Suprarenal Capsules, *Jour. Physiol.*, 1895, xviii, 231; *Proc. Physiol. Soc.*, *Ibid.*, 1894.
- <sup>4</sup>Szymonowicz, L.: Die funktion der Nebenniere, *Pflüger's Arch.*, 1896, lxiv, 97.
- <sup>5</sup>Abel, J. J.: On Epinephrin, the Active Constituent of the Suprarenal Capsule, *Am. Jour. Physiol.*, 1899, ii, 3.
- Abel and Crawford: On the Blood Pressure Raising Constituent of the Suprarenal Capsule, *Bull. Johns Hopkins Hosp.*, 1897, 76.
- <sup>6</sup>Aldrich, J. B.: A Preliminary Report on the Active Principle of the Suprarenal Gland, *Am. Jour. Physiol.*, 1901, v, 457.
- <sup>7</sup>Takamine, J.: The Isolation of the Active Principle of the Suprarenal Gland, *Jour. Physiol.*, 1901, xxvii, 29; *Am. Jour. Pharm.*, 1901, lxiii, 523.
- <sup>8</sup>von Fürth, O.: Zur kenntniss der brenczatechinähnlichen substanz der Nebennieren, *Ztschr. f. physiol. Chem.*, 1898, xxvi, 15.
- <sup>9</sup>Dakin, H. D.: The Synthesis of a Substance Allied to Adrenalin, *Proc. Royal Soc.*, London, 1905, lxxvi, 491.

- <sup>10</sup>Lewandowsky, M.: Ueber die Wirkung des Nebennierenextraktes auf die glatten Muskeln, im besonderen des Auges, Arch. f. Anat. u. Physiol., 1899, 360.
- <sup>11</sup>Langley, J. N.: Observations on the Physiological Action of Extracts of Suprarenal Bodies, Jour. Physiol., 1901, xxvii, 237.
- <sup>12</sup>Elliott, T. R.: The Action of Adrenalin, Jour. Physiol., 1905, xxxii, 401.
- <sup>13</sup>Cannon and de la Paz: Emotional Stimulation of Adrenal Secretion, Am. Jour. Physiol., 1911, xxviii, 64.
- <sup>14</sup>Stewart, G. N.: So-called Biological Tests for Adrenalin in Blood with Some Observations on Arterial Hypertonus, Jour. Exper. Med., 1911, xiv, 377.
- <sup>15</sup>Hoskins, R. G.: A Consideration of Some Biological Tests for Epinephrin, Jour. Pharm. and Exper. Therap., 1911, iii, 93.
- <sup>16</sup>Stewart and Rogoff: The Proportion in which Adrenalin Distributes Itself between Corpuscles and Serum in Relation to the Technique of Testing for Epinephrin in Blood, Jour. Pharm. and Exper. Therap., 1917, ix, 393.
- <sup>17</sup>Dreyer, G. P.: On Secretory Nerves to the Suprarenal Capsule, Am. Jour. Physiol., 1899, ii, 203.
- <sup>18</sup>Tscheboksaroff, M.: Ueber Sekretorische Nerven der Nebennieren, Pflüger's Arch., 1911, cxxxvii, 59.
- <sup>19</sup>Stewart, G. N.: The Alleged Existence of Adrenalin (Epinephrin) in Pathological Sera, Jour. Exper. Med., 1912, xv, 547.
- <sup>20</sup>Meltzer, S. J.: Studies on the "Paradoxical" Pupil Dilatation Caused by Adrenalin, Am. Jour. Physiol., 1904, xi, 37.
- <sup>21</sup>Joseph and Meltzer: The Effect of Stimulation of the Peripheral End of the Splanchnic Nerves upon the Pupil, Proc. Am. Physiol. Soc., Am. Jour. Physiol., 1912, xxix, 34.
- <sup>22</sup>Asher, L.: Die innere Sekretion der Nebenniere und deren Innervation, Ztschr. f. Biol., 1912, lviii, 274.
- <sup>23</sup>Elliott, T. R.: The Control of the Suprarenal Glands by the Splanchnic Nerves, Jour. Physiol., 1912, xlv, 374.
- <sup>24</sup>Stewart, Rogoff and Gibson: The Liberation of Epinephrin from the Adrenal Glands by Stimulation of the Splanchnic Nerves and by Massage, Jour. Pharm. and Exper. Therap., 1916, viii, 205.
- <sup>25</sup>O'Connor, J. M.: Ueber die Abhängigkeit der Adrenalinsekretion vom Splanchnicus, Arch. f. exper. Path. u. Pharmakol., 1912, lxxviii, 383.
- <sup>26</sup>Trendelenburg, P.: Über die Adrenalin-konzentration im Saugtierblut, Archiv. f. exper. Path. u. Pharmakol., 1915, lxxix, 154.
- <sup>27</sup>Stewart and Rogoff: The Spontaneous Liberation of Epinephrin from the Adrenals, Jour. Pharm. and Exper. Therap., 1916, viii, 479.
- <sup>28</sup>Stewart and Rogoff: Quantitative Experiments on the Liberation of Epinephrin from the Adrenals after Section of Their Nerves, with Special Reference to the Question of the Indispensability of Epinephrin for the Organism, Jour. Pharm. and Exper. Therap., 1917, x, 1.
- <sup>29</sup>Cannon and Hoskins: The Effects of Asphyxia, Hypernea and Sensory Stimulation on Adrenal Secretion, Am. Jour. Physiol., 1911, xxix, 274.
- <sup>30</sup>Ott and Scott: The Action of Glandular Extracts upon the Amount of Epinephrin in the Blood, Jour. Pharm. and Exper. Therap., 1912, iii, 625.
- <sup>31</sup>Stewart and Rogoff: The Relation of the Rate of the Spontaneous Liberation of Epinephrin to the Rate of Blood Flow through the Adrenals, Am. Jour. Physiol., 1917, xlv, 149.
- <sup>32</sup>Stewart and Rogoff: The Influence of Asphyxia upon the Rate of Liberation of Epinephrin from the Adrenals, Jour. Pharm. and Exper. Therap., 1917, x, 49.
- <sup>33</sup>Stewart and Rogoff: Effect of Stimulation of Sensory Nerves upon the Rate of Liberation of Epinephrin from the Adrenals, Jour. Exper. Med., 1917, xxvi, 637.
- <sup>34</sup>Cannon, W. B.: A Note on the Effect of Asphyxia and Afferent Stimulation on Adrenal Secretion, Science, 1917, xlv, 463.
- <sup>35</sup>Gley and Quinquaud: La sécrétion surrénale d'adrénaline ne tient pas sous sa dépendance l'effet vaso-constricteur du sang asphyxique, Compt. rend. Soc. de biol., 1917, lxxx, 15.
- <sup>36</sup>Stewart and Rogoff: The Influence of Certain Factors, Especially Emotional Disturbances, on the Epinephrin Content of the Adrenals, Jour. Exper. Med., 1916, xxiv, 709.
- <sup>37</sup>Cannon, W. B.: The Emergency Function of the Adrenal Medulla in Pain and the Major Emotions, Am. Jour. Physiol., 1914, xxxiii, 356.
- <sup>38</sup>Stewart and Rogoff: The Alleged Relation of the Epinephrin Secretion of the Adrenals to Certain Experimental Hyperglycemias, Am. Jour. Physiol., 1917, xlv, 543.
- <sup>39</sup>Fulk and Macleod: Evidence that the Active Principle of the Retroperitoneal Chromaffin Tissue has the Same Physiological Action as the Active Principle of the Suprarenal Glands, Am. Jour. Physiol., 1916, xl, 21.
- <sup>40</sup>Bedford, E. A.: The Epinephric Content of the Blood in Conditions of Low Blood Pressure and Shock, Am. Jour. Physiol., 1917, xlii, 235.
- <sup>41</sup>Stewart and Rogoff: The Relation of the Spinal Cord to the Spontaneous Liberation of Epinephrin from the Adrenals, Jour. Exper. Med., 1917, xxvi, 613.



## THE RESPONSIBILITY OF THE VACCINATOR IN OVERCOMING THE RATIONAL OBJECTIONS TO SMALLPOX VACCINATION\*

JOHN NIVISON FORCE, M.D., GR., P.H., AND IDA MAY STEVENS, M.A.(P.H.),  
BERKELEY, CAL.

EDWARD JENNER published his "Inquiry into the Causes and Effects of Variolæ Vaccinæ" in 1798. Two years later, smallpox vaccine was brought to this country, and Waterhouse of Cambridge, Massachusetts, performed the first American vaccination on his son, aged five years. By 1803, the practice of vaccination had spread throughout Europe and a ship had been dispatched to carry "the blessed vaccine" to the Spanish possessions in the Old and New Worlds.

In contrast to the remarkable support which the new discovery received throughout the world, we find an antivaccination propaganda based on the belief that those inoculated with cowpox would assume bovine characters and features. In a pamphlet entitled "Cowpox Inoculation no Security against Smallpox Infection," a Doctor Rowley makes the following statement: "Various beastly diseases common to cattle have appeared among the human species since the introduction of cowpox—cowpox mange, cowpox abscess, cowpox ulcer, cowpox gangrene, cowpox mortification, and enormous hideous swellings of the face, resembling the countenance of an ox with the eyes distorted and the eyelids forced out of their true situation. Smallpox is a visitation from God, but the cowpox is produced by presumptuous man; the former was what Heaven ordained, the latter is, perhaps, a daring violation of our holy religion."

In an age when "laudable pus" accompanied almost every surgical operation, it is not remarkable that many severe infections followed vaccination, especially if the large scarifications, so vigorously condemned by Jenner, were used. The superstition of the transmission\* of animal characters is very old and finds a present-day echo in the statement that the victim of rabies "barks like a dog." It is not customary in this generation to blame an offended deity for private and public calamity, but many people, misguided by popular advertisements, are pouring libations in the form of disinfectants into the garbage can and house plumbing, thinking thereby to purchase immunity from disease. These are the people who say that smallpox has decreased because of better sanitation.

Setting aside the unscientific objections to vaccination, which have only slightly changed their characteristics in the past century, let us consider the rational objections to vaccination, which we find even among persons medically orthodox. These objections are two in number and the responsibility for their continuance rests squarely on the medical profession.

### THE USE OF SMALL MULTIPLE SCARIFICATIONS

The first objection to vaccination is that it is dangerous since it is often

---

\*From the Laboratory of Hygiene, University of California, Berkeley, Cal.



accompanied by a secondary infection which results in a slowly granulating ulcer and a disfiguring scar. In a previous communication,<sup>1</sup> we have shown that "the prevention of secondary infection following smallpox vaccination depends essentially on the use of small multiple scarifications which, when inoculated, give rise to small vesicles which are not easily broken, and do not foster anaerobic conditions and a central slough." The large unsightly vaccination scars so commonly seen are due to the slow healing of an ulcer produced by the inoculation of a large cross-scarified area. *The vaccine colony grows only in unbroken skin.* It is obvious, therefore, that vaccinators should employ the smallest scarification consistent with the implantation of the vaccine on the derma. It is our custom to remove three small circles of epidermis by the rotation of a chisel having a 2.0 mm. cutting edge, but excellent results are obtained by short scratches made at least 2.0 cm. apart with an ordinary sewing needle. Hill<sup>2</sup> advocates placing a drop of vaccine on the skin, and carrying a small portion through to the derma by acupuncture. As long as small scarifications are used, neither the method of vaccine preparation, the bacterial content of the vaccine, or the use of antiseptics on the resulting vesicle will influence the course of a primary vaccinia.

#### REPEATED FAILURES TO SECURE SUCCESSFUL VACCINATION ARE NOT EVIDENCES OF IMMUNITY

The second and more serious objection to vaccination is that it does not protect against smallpox. As early as 1804, Jenner was forced to admit that smallpox could occur in persons who had previously been vaccinated, though he believed that the failure to secure immunity was the result of improper technic. The presence of a large irregular scar following vaccination is only slightly better evidence of immunity than its continual absence after repeated unsuccessful attempts to vaccinate. In the first instance the scar may be the result of an infective process which has destroyed the vaccine organism before immunity has been produced, and in the second instance the failure to produce a vaccinia may be due to the lack of potency in the vaccine employed.

The practice of issuing vaccination certificates based on a "guess" that the subject is immune because, one, two, or three vaccinations fail to take is almost as potent for evil as improper vaccination technic. Every year we produce primary vaccinias in persons with a history of from one to four or more failures previous to entering the university (Table I). Every year we produce primary vaccinias in persons with a history of having recently swallowed "variolin" pills. Experiences of this sort have bred within us an intense skepticism concerning vaccination certificates unaccompanied by vaccination scars. We do not question the motives behind the issue of these certificates to the scarless, but our experience shows that persons with and without vaccination scars are constantly being declared immune where no immunity exists. As shown in Table II, only 51 (38 per cent) of 136 persons whose vaccination scars were more than twenty years old gave evidence of immunity on revaccination, while 46 (14 per cent) of 318 persons whose vaccination scars were less than ten years old gave unmodified vaccinias. If these people get smallpox, there is more

fodder for the nourishment of the antivaccination propaganda. Who is to blame for this condition if not the man who "guesses" that his patient is immune? Among our previously unvaccinated intrants with no history of smallpox, we have 100 per cent of vesicle formation on vaccination. We have yet to see our first natural immune. We do not believe that he exists. Repeated vaccination with an inert vaccine will occasionally render the subject immune to potent vaccine. These persons present no vaccination scar, but show a well-marked reaction of immunity on revaccination. On the other hand, a failure will be characterized by the absence of any reaction at the vaccination site other than that due to scarification trauma. True failures furnish valuable checks on vaccine potency.

Inquiry at the laboratory, following 23 failures with a certain vaccine disclosed the fact that the air used in driving the ether out of this vaccine had been accidentally saturated with a chemical fatal to the vaccinia organism. Brooks<sup>3</sup> reports failure of vaccine to act in two hundred persons in Oxford, New York, during a smallpox outbreak in 1915. This failure was traced to contact with steam pipes during the transit of the vaccine.

In order to test the effect of age, method of preparation, and method of storage on the potency of vaccine, we conducted a number of vaccinations with vaccines of various ages prepared by various methods.

In a series of vaccinations made with vaccines ground in glycerine and stored in the ice box for varying periods before use, there were eight reactions and no failures at the end of six months storage; eight reactions and one failure at the end of five months; eighteen reactions and one failure at the end of four months; twenty-four reactions and one failure at the end of three and one-half months; one hundred seventy-eight reactions and one failure when the vaccine had been stored for five weeks; and thirty-three reactions with no failures when the vaccine had been stored two weeks before use.

In a similar series made with a glycerinated vaccine which had in addition been subjected to the action of ether to reduce the number of contaminating organisms before storage in the ice box, there were eight reactions and one failure with vaccine six months in storage; two reactions and four failures with vaccine held five months; nineteen reactions and no failures with vaccine held four months; and fourteen reactions with no failures with vaccine held three and one-half months. The percentage of failures in this series is only slightly higher than with the corresponding glycerinated vaccines which had not been etherized.

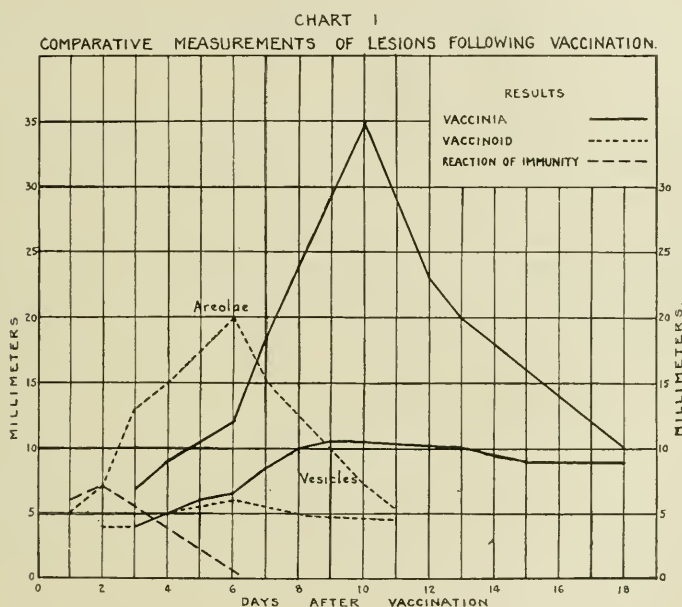
In a series of 230 vaccinations made with a vaccine which had been etherized but showed a high bacterial count there were no failures; but equally good results were obtained in 168 vaccinations with a vaccine which had been etherized until practically sterile.

In thirty-eight vaccinations with a vaccine which had been incubated for twenty-four hours after grinding in glycerine and then stored in the ice box until practically sterile, there were no failures.

A series of sterile vaccines prepared according to the method of Noguchi,<sup>4</sup> by passage through rabbit testicles, resulted in five reactions and four failures

on second passage of the strain originally received from the Rockefeller Institute; ten reactions and eleven failures on the fifteenth passage; and seven reactions and fifty-six failures on the twenty-ninth passage through rabbit testicles. This shows a steady deterioration which Greely<sup>5</sup> believes to be due to the absence of certain spore-like forms of the organism which are present in vaccine cultivated on the skin.

The results of these vaccinations show that the greatest factor in vaccine potency is the maintenance of ice box temperature during the entire period between manufacture and use. Etherizing and preliminary incubating do not appear to affect the potency provided the vaccine is properly iced. On the other hand, failure to secure constant ice box conditions will result in the use of large amounts of inert virus even if the expiration date is not exceeded. With



no check on the potency of the vaccine, it is obviously absurd to issue a certificate that the vaccinator has used due diligence and can not produce a successful vaccination.\*

#### COMPARISON OF THE THREE TYPES OF SUCCESSFUL VACCINATION

Depending on the response of the subject to the invading vaccine antigen, we are able to distinguish three forms of successful vaccination. When antibodies are present in the tissues, the invading organisms are at once destroyed. This reaction of immunity is characterized by an areola which appears within twenty-four hours after the vaccination and fades out without developing into a vesicle.

\*Since the above was submitted for publication, I have observed two true failures in previously unvaccinated persons, with a vaccine three months old, obtained from an establishment whose product we have found to give uniformly good results when six months old. In the first instance the vaccine reached our icebox via an Army Supply Depot, and in the second it was transferred from the manufacturer's icebox directly to ours. This emphasizes the need for icebox or vacuum bottle conditions at all times from establishment to arm.—J. N. F.

Inert vaccine may produce the reaction of immunity in an immune subject, or even a slight papule on the fourth or fifth day in a nonimmune subject. When antibodies are no longer present but the ability to quickly form them still exists, the growing colony of vaccinia organisms is arrested and destroyed before it reaches its full development. In consequence, on the fifth day following vaccination this secondary vaccinia (vaccinoid) is characterized by a larger areola and a smaller vesicle than a primary vaccinia of the same age.

The characteristics of the three types of successful vaccination may be shown by plotting the daily average measurements of the lesions of a series of vaccinas with corresponding measurements of a series of vaccinoids and a series of reactions of immunity (Chart I). The vaccination certificate issued by this university does not provide for vaccination failure. The reaction of immunity is a successful vaccination because the act of calling out antibodies to produce this reaction brings up immunity to its maximum. Smallpox immunity is like a clock: the further it has run down the longer it takes to wind it; but it can only be wound to a certain point no matter where you start winding. Producing the reaction of immunity is like winding an eight-day clock which has only run one day since the previous winding. You would not say that you had failed to wind the clock because you had to stop after one or two turns of the key.

The secret of our 100 per cent of vesicle formation in the previously unvaccinated (Table I) is simple. It consists in the two words "fresh" and "cold" applied to the smallpox vaccines. Fresh because it is the very latest released by the manufacturer, cold because it is barely half an hour from his ice box to our ice box and is kept in cracked ice while in the vaccination room. Any vaccinator who will buy his smallpox vaccine in bulk, have it shipped in a vacuum bottle to avoid possible steam pipe episodes, and keep it at ice box temperature after removal from the vacuum bottle, has a right to demand 100 per cent of vesicle formation in the previously unvaccinated. If one of these subjects gives a reaction of immunity on first vaccination, you may rest assured that smallpox has been there before you.

TABLE I.

RESULTS OF VACCINATION OF UNIVERSITY OF CALIFORNIA INTRANTS DURING THE ACADEMIC YEAR 1916-17 CLASSIFIED ACCORDING TO VACCINATION HISTORY.

	PRIMARY VACCINIA		SECONDARY VACCINIA (VACCINOID)		REACTION OF IMMUNITY	
	NO.	%	NO.	%	NO.	%
<i>Scar absent</i>						
Previously unvaccinated	178	96	8	4		
History of smallpox	7	24	11	38	11	38
History of ingestion of "variolin"	2	100				
Previously vaccinated but no scar						
once	145	92	10	6	2	2
twice	37	92	2	5	1	3
three times	24	86	4	14		
four times or more	29	88	3	9	1	3
<i>Scar indistinct</i>	16	52	10	32	5	16
<i>Scar distinct</i>	7	12	28	39	30	49



TABLE II.

RESULTS OF THE REVACCINATION OF 898 PERSONS SHOWING VACCINATION SCARS.

AGE OF SCAR	PRIMARY VACCINIA		SECONDARY VACCINIA (VACCINOID)		REACTION OF IMMUNITY	
	NO.	%	NO.	%	NO.	%
Under 10 years	46	14	121	38	151	48
10 to 20 years	61	14	171	38	212	48
Over 20 years	48	35	37	27	51	38

## CONCLUSIONS

1. The use of small multiple insertions will shorten the course and minimize the dangers of smallpox vaccination.

2. Properly cooled smallpox vaccine produced a high percentage of successful vaccinations four, five, and six months after collection.

3. The best results were secured with vaccine used within one month after collection.

4. Vaccine prepared according to the method of Noguchi, while bacteria-free, seems impracticable for commercial use on account of rapid deterioration.

5. Since the object of vaccination is to secure immunity, successful vaccination consists in producing either a primary vaccinia, a secondary vaccinia (vaccinoid), or a reaction of immunity (Chart I).

6. All vaccination certificates should be based on actual evidence of immunity as indicated by one of the three reactions described, and not on repeated failures to secure a typical vaccinia.

7. There will be no vaccination failures if cold, fresh, potent smallpox vaccine be used.

## BIBLIOGRAPHY

- <sup>1</sup>Force and Stevens: Some Factors Alleged to Influence the Duration and Severity of Vaccinia, *Jour. Am. Med. Assn.*, 1917, lxxviii, 1247.  
<sup>2</sup>Hill: Acupuncture: The Best Method of Vaccination, *Am. Jour. Public Health*, 1917, vii, 301.  
<sup>3</sup>Brooks: The Quarantine Control of Contacts, *Health News*, 1916, xi, 289.  
<sup>4</sup>Noguchi: Pure Cultivation in Vivo of Vaccine Virus Free from Bacteria, *Jour. Exper. Med.*, 1915, xxi, 539.  
<sup>5</sup>Greely: Cultivation of Organisms of Vaccinia, Variola, and Varicella, *Med. Rec.*, New York, 1916, xc, 165.

# WAR DEAFNESS AND ITS PREVENTION—A REPORT OF TESTS UPON EIGHT PREVENTIVES\*

BY STACY R. GUILD, ANN ARBOR, MICH.

THE object of the series of experiments here reported was to test the relative efficiency of various devices for preventing injuries to the ear parts by detonations. Eight preventives have been used in the work done to date; as others are obtained they will be tested in similar manner.

## PREVENTIVES USED IN TESTS

The first two devices were sent to me by Major V. C. Vaughan, of the National Research Council, with the request that I try them out and report. They had been submitted to the Council by the inventors.

1. The "Scientific Ear Drum Protector 'Tommy,'" patented in Great Britain and France in 1915, manufactured by Geo. F. Berry, 4 Cullum Street, Fenchurch Street, London, E. C. It consists of a hollow soft rubber spherical bulb with an opening on one side surrounded by a flange. The external diameter of the spherical part is about 9.5 mm., and of the flange 11.5 mm. A pair in an envelope with a short rod for helping to insert them properly retails at one shilling.

2. A device invented, according to the letter from Major Vaughan, by Dr. J. Gordon Wilson, Professor of Otology at Northwestern University, and at present in the medical corps of the British army, and Professor A. A. Michelson, of the Physics Department of the University of Chicago. The device consists of a hollow framework of hard rubber in which is supported a valve of light metal so arranged as to stay open and permit ordinary sounds to pass at the edges, but so adjusted that detonation waves can cause it to shut by moving inward and forming contact with what may be called the valve-seat. The outer end is covered by a fine meshed wire gauze placed in a detachable metal cap. The large end is 17 mm. in diameter; the small end is 8 mm. at the center of a slight bulge near the end. This inner end is too small to fit any of the meatus I have tried it in, but this is a matter easily remedied. It is probable, judging from the fact that the end is small and that one of the two instruments sent was embedded in a cast of plaster of Paris made to fit some person's right ear, that the intention was to fit each user individually by making casts for each.<sup>1</sup> This would certainly be difficult, if not impracticable, to carry out successfully in an army, for the casts would require frequent renewal. Two other serious objections are evident on critical consideration of this instrument. In field service, especially in trench life, with its mud and other dirt, no wire gauze would be able to prevent particles of material from passing and a very small particle would be sufficient, when caught under the edge of the valve, to hold it open permanently. The second

\*From the Department of Anatomy, University of Michigan Medical School, Ann Arbor, Mich. A previous paper on this subject may be found in this JOURNAL, ii, 849.

<sup>1</sup>The device submitted in a cast does not open into the external meatus, but is so placed that it must have rested against the concha just posterior to the orifice of the meatus.

objection is the one I raised in the previous article as being common to all hard obturators; namely, that bullets passing alongside the head and wounding only the pinna or the outer part of the external meatus, in themselves relatively slight wounds and of quite common occurrence, would shatter the hard parts of an obturator present and form secondary projectiles of the fragments, causing much more severe wounds. A further theoretical objection is that there is nothing to prevent the air in the meatus from passing out during the rarefaction phase following the condensation phase of the detonation wave; the valve acts only in one direction. This would permit injury due to the air contained in the middle ear cavity, the eustachian tube not being open normally except during part of the swallowing act.<sup>2</sup>

The next two devices were obtained by direct purchase from the manufacturers.<sup>3</sup> For a description and critical discussion of them and the following preventive measures the reader is referred to the previous article.

3. The Elliott "Perfect Ear Protector," manufactured by J. A. R. Elliott, P. O. Box 201, New York City.

4. The "Mallock-Armstrong Ear Defender," 1916 pattern, manufactured by the Mallock-Armstrong Ear Defender Co., 2, Palmer Street, Westminster, London, S. W. This 1916 pattern differs from the description of the device given in the previous article in that the diaphragm between the wire gauzes is not of metal, as the 1915 patent claims call for, but appears to be of a membranous nature, and is translucent. The device is now made in seven sizes instead of four, to enable more exact fitting. The price has been advanced from three shillings to four shillings a pair.

5. Wax cones which I have made after the description given by Rho as having been adopted in the Italian navy.

6. Dry cotton, placed firmly.

7. Cotton saturated with glycerine, carefully kneaded to drive out all the air bubbles possible. Before using, the excess glycerine was squeezed out with the fingers.

8. Cotton saturated with vaseline, carefully worked in until a uniform mass was obtained.

#### METHOD OF TESTING

The mechanical devices are made only of a size to fit human ears, and so the ideal manner of testing them is upon human subjects, which will be, of course, the final test of efficiency. But for laboratory tests, in which autopsies and histologic preparations are desired, animals must be used. To do this, it was necessary to devise some new apparatus. To have used a plain piece of tapering glass tubing drawn to a size at one end suitable for fitting the meatus of the animal used and large enough at the other end to receive the various devices would have been the simplest way, but a very serious objection is met with due to the fact that the consistency of the glass differs so much from the pliable human ear that

<sup>2</sup>I wish at this place to express my great admiration for the work of both Prof. Michelson and Dr. Wilson in their chosen fields; I have hesitated to express criticisms of their instrument, especially so since mechanics is not my own field.

<sup>3</sup>I ordered also three pairs of the Rogers Plastic Ear Plugs, described in the previous article, but have not yet succeeded in obtaining them, though they were ordered from London in June.

a fair test of the holding power of the preventives would not be given. A plain piece of rubber tubing attached to the glass tube simulates the consistency well enough, but not the shape of the part of the human external ear in which the devices are intended to fit. Two of the devices, the "Tommy" and the Elliott protector, have flanges which fit beautifully over the end of a piece of tubing, but not so well in the actual ear, where the anterior wall of the meatus is continuous directly with the posterior surface of the tragus, while a "shoulder" is present at the posterior part of the orifice. The idea was conceived, after trials with these other things, of trying to make a holding device of soft rubber of the shape of the concha and first part of the meatus of a human ear. Such a device would, if made with sufficiently thick walls, be of a consistency simulating the human ear in the part holding the devices, and would, therefore, give a better test of them. Being inexperienced in working with rubber, I consulted Dr. E. L. Whitman, Instructor in Dental Technics in the Dental School of the University. He said that my idea could be carried out, and I was able to interest a dental student, Mr. G. C. Lubke, who made for me two rubber "ears," which answer the requirements very well. These were built over a plaster cast made of an ear of a cadaver. A short extension of the outer part of the meatus in the form of a very heavy walled rubber tube, built as an integral part of the device, provides a means of fastening a glass tube for connecting to the external meatus of animals.

Glass tubes may, of course, be made to fit any animal it is desired to use, being simply glass tubing drawn, or blown, to the shape and size needed. For guinea pigs, which I have used in these experiments, I made several of these plugs of varying sizes. The one which I like best after experience with them is 17.5 mm. long, and has an internal diameter of 6 mm. at the large end, tapering to 3.5 mm. at the smallest part. The small end was flanged slightly in making, so that the 3.5 mm. diameter is about 1 mm. from the end. This tube does not pass into the meatus of a guinea pig, but fits over the orifice, and in order to completely close any small openings left a soft wax was used. This was made by mixing beeswax and vaseline in about equal parts by heating. The wax was packed about the glass tube and built up in such a way that it formed a wall that continued the outer surface of the rubber and extended to the side of the head of the animal. Those tubes small enough to enter the guinea pig meatus have an internal diameter so small that a considerable degree of protection is afforded by the tubing itself. (See Table II.)

In order to use such an arrangement successfully, it is essential that either the apparatus be so fastened to the animal that it will follow all the movements it may make without becoming loosened, or that the head of the animal be immobilized. The latter procedure seemed preferable and as I knew of no apparatus suitable for this purpose a new one had to be made. It consists of a base of wood 10 cm. wide by 4 cm. thick by 35 cm. long, on which two strips of canvas 6 cm. wide are tacked. These strips run across the base and at either end serve as hinges to which are attached the side pieces, each of which is made of thin narrow strips of wood nailed together to form a latticework 23 cm. long by 8 cm. wide. When closed, the base and side pieces form a space with a triangular cross section 4 cm. wide at the base and nearly 8 cm. high; the spaces of the lat-



ticework permit parts of the body of an animal to bulge. To further immobilize the head, on either side an obliquely placed bar of wood was attached to the inner side of the lower strip of the latticework near one end. These fit behind and under the angle of the jaw, and, being movable, can be adjusted readily to the size of the animal. To prevent vertical movement of the front of the head, a completely removable crossbar of wood is fastened under the front part of the lower jaw; this is also adjustable by means of notches in the latticework. When properly adjusted, the ears of a guinea pig being held are well exposed between strips of the side pieces. The apparatus of the dimensions given is adaptable to guinea pigs ranging from 200 to 400 grams. Experience has shown that when one sets the "jaw pieces" of this holding apparatus tight enough to completely immobilize the head of an animal the external auditory canal is liable to be partly or even totally pinched shut. When set loose enough to assure an open canal a slight amount of movement is possible; it was found that most animals did not try to move after the first struggles while fastening them in. A few animals moved after packing of the wax and required repacking; only one animal out of some fifty used persisted in moving so much that it was impossible to satisfactorily place a packing. In every case, in order to check up on the loosening of the packing and a possible entry of the detonation wave thereby, the wax was examined carefully after the shooting, and the condition of it has been made a matter of record in the tabulated results.

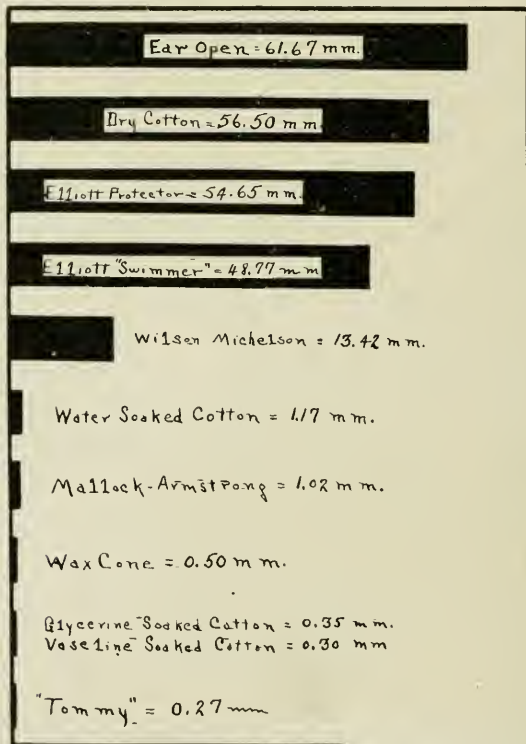
The source of the detonation wave used was the firing of a 45 caliber Colt automatic pistol, using Peters' ammunition. The experiments were conducted in the open at a place where a hillside forms a suitable stop for bullets. While the detonation is not of the initial intensity of that caused by an exploding shell or by the firing of a large artillery piece, yet by using the relatively small detonation at a short distance the force is doubtless approximated to that in the zone about a larger explosion in which ears are injured without causing death by "shell shock." A series of controls showed 15 cm. to be a suitable distance, the tympanic membrane being regularly ruptured with coagulated blood and edema present in the mucous membrane at autopsy.

Submitting animals to large gun and shell detonations might be done at some of the munitions proving grounds and at certain government works, if proper authority and some laboratory facilities were provided, and such tests would be an interesting check on these.

The routine procedure for each experiment was as follows: (1) The hair projecting over the ear to be protected (the left ear was used in all cases) was trimmed with scissors; (2) the animal was fastened in the holding apparatus and the apparatus adjusted so as to be sure the external auditory canal was not pinched shut; (3) the animal in the apparatus was then turned on one side and the glass tube, with the large end already inserted in the rubber ear in which the protecting device to be tested had been carefully adjusted, was placed over the meatus (or inserted in it in the case of the small tubes used a few times) and the soft wax mixture carefully packed as already described above; (4) a cord passed through the rim of the rubber ear was fastened to the framework of the apparatus in such a way as to support the weight of the ear and prevent it from

becoming loosened by gravity when the animal and apparatus are placed upright again; (5) the apparatus and contained animal were placed on the ground selected, the muzzle of the gun held at the predetermined point giving the desired distance and direction and the shot fired; (6) the condition of the wax packing was noted immediately and the animal released as soon as possible.

The damage to the ear by detonations has been discussed fully in the previous article. The variations from normal that indicate injury to the hearing are to be sought for in two places in animals, (1) the middle ear parts and (2) the cochlear parts. Clinical tests on the hearing ability of animals are very unsatisfactory when a total loss of hearing has not occurred, and that even this is dif-



A graphic presentation of the results with the 44 caliber revolver.

icult to determine is evidenced by the heated discussion from 1892 to 1895 as to whether animals with the eighth cranial nerve severed some time previously could hear. The experienced laboratory worker, W. Wundt, was deceived, among others; with such a record of the interpretation of hearing tests, I am depending entirely upon organic injuries to judge the damage done to the hearing. The condition of the middle ear parts may be observed with the unaided eye at autopsy; the condition of the cochlear parts can be told only by microscopic examination preceded by careful histologic preparation. Yoshii has stated that the maximum degree of change in the cochlear parts is reached on the second to third day after the injurious detonation. He used only animals exposed to the

full force of a detonation. For this reason I killed the animals used about forty-eight hours after exposure.

In a series of experiments conducted during the past two years I have been trying to find the best technic for handling cochleæ, and have tried most of the procedures recommended by those who have reported work on cochleæ and also some new variations. This work was done preliminary to experiments on the physiology of hearing, which the war has postponed indefinitely, but which I hope at some future time to carry out. At that time the results of the various technical procedures will be fully considered. For the present it suffices to say that in these experiments I am using the method which has given me the best general results. It will be given in full in the report of the condition of the cochleæ of these animals submitted to detonations. As the method includes fixation of the tissue by vascular injection with a preliminary thorough washing out of the blood by physiologic saline solution, inflammatory changes in the middle ear could not be judged by the coloration at autopsy, but had to be judged only by the presence of coagulate and edema. The significance of the loosening of the mucous membrane was not recognized by me in the first animals used and accordingly it was only occasionally recorded at first. It was not until a group of control animals that had not been exposed to detonations was examined that I became certain that the loosening of the mucous membrane was not an artefact due to the injection procedure. This accounts for the blanks in the records of some of the animals with regard to the presence of edema. The condition of the tympanic membrane is not affected by the injection and may be directly observed, as may also the presence of areas of coagulate which are not removed by the washing out procedure.

#### REASON FOR DIVIDING THE REPORT

Since the handling of the cochleæ by the method used, preparatory to sectioning, requires at the very least 85 days, and the first animals were used September 5, it has seemed advisable to report the observations on the middle ear parts without waiting for those on the cochlear parts, especially since the results are positive, certain devices having protected the middle ear parts better than others, and since the injury to ears of soldiers is occurring every day. The study of the sections of the cochleæ, when prepared properly, should give more evidence, but its nature can not be predicted. It may show marked differences between degree of protection afforded by those devices which best protected the middle ear parts. Due to the time required for sectioning, staining, and studying them, and teaching duties, it will probably be late next spring before I can report the cochlear condition.

#### OBSERVATIONS ON THE MIDDLE EAR PARTS

The observations are presented in tabulated form; the tables showing the control experiments being placed before those of the preventive measures tested. The items shown in the tables need no other explanation than that already given above.

The finding of normal middle ear parts in one side of No. 17 and No. 18,

TABLE I  
CONTROL EXPERIMENTS

ANIMALS WITH BOTH EARS OPEN; NO APPARATUS IN EITHER EAR

GUI-NEA PIG NO.	DISTANCE OF NEAREST EAR FROM MUZZLE	NO. OF SHOTS FIRED	SIDE	CONDITION OF TYMPANIC MEMBRANE AT AUTOPSY	AMOUNT OF COAGULATE IN AND ON MUCOUS MEMBRANE OF THE MIDDLE EAR	EVIDENCE INDICATING EDEMA OF THE MUCOUS MEMBRANE	REMARKS ESPECIALLY ON THE POSITION OF THE PISTOL
45	60 cm.	1	Right	Intact — radial streak of coagulate	Small	Slightly loosened	Shooting from above and in front of animal downward at 20° angle with the horizontal
			Left	Intact	One large area	Slightly loosened	
44	30 cm.	1	Right	About $\frac{1}{3}$ of area broken	Medium	Loosened	Shooting from above and in front downward about 20° slightly along left side
			Left	Whole center gone	Small	Loosened	
16	15 cm.	1	Right	Off along most of circumference	Medium		From above and behind directly over animal
			Left	Not quite as bad as right side	Medium	Loosened	
17	15 cm.	5	Right	Intact	None	None	(See text matter below) From above and in front along right side of animal
			Left	Completely gone	Large		
18	5 cm.	3	Right	Intact	None	None	(See text matter below) From above and in front along left side
			Left	Completely gone	Large		
39	10 cm.	2	Right	Off of $\frac{3}{4}$ of circumference	Medium	Loosened	Shooting from directly above
			Left	Fringes only left	Large	Loosened	

after shots fired at so short a distance, is to be interpreted only on the basis of the external canal having been pinched shut by the jaw pieces of the holding apparatus. These cases were among the first animals used and thereafter special precaution was taken to prevent repetition of this condition, as has been mentioned in the description of the routine procedure. For a short time after the shooting, the animals appeared to be somewhat dazed, but none were unconscious when released. The last one listed above was not placed in the holding apparatus and it was able to run away after each shot; it did not stagger, but seemed somewhat dazed.

The controls on the possible protection afforded by the apparatus used to hold the preventive devices show clearly that there is an approximately equal damage to the middle ear parts with and without the rubber "ear" and glass tube in place, when using tubes 2.5 mm., and upwards, in internal diameter at the smallest part. With the 2 mm. tube, a certain degree of protection may be



TABLE II  
CONTROL EXPERIMENTS

ANIMALS WITH GLASS TUBE AND RUBBER "EAR" (WITHOUT ANY PREVENTIVE DEVICE INSERTED) IN ONE EAR AND THE OTHER EAR OPEN

GUL-NEA PIG NO.	SIDE	SMALL- EST DI- AMETER OF THE GLASS TUBE IN MM.	CONDITION OF WAX PACKING AFTER SHOOTING	CONDITION OF TYMPANIC MEMBRANE AT AUTOPSY	AMOUNT OF COAGULATE IN AND ON MUCOUS MEMBRANE OF THE MID- DLE EAR	EVIDENCE INDICAT- ING EDEMA OF THE MUCOUS MEMBRANE	REMARKS ESPECIALLY ON THE POSITION OF THE PISTOL
19	Right	3.5	O.K.	Ruptured badly	Medium		Two shots — one at 10 cm. from each ear backward along sides
	Left			Ruptured badly	Medium		
52	Right			Off on $\frac{3}{4}$ of circumfer- ence	Large	Slightly loosened	Usual ar- rangement*
	Left	3.5	O.K.	Fringe	Large	Badly loosened	
51	Right			Fringe	Large	Slightly loosened	Usual ar- rangement*
	Left	3.1	O.K.	Fringe	Large	Slightly loosened	
50	Right			Fringe	Large	Loosened	Usual ar- rangement*
	Left	2.5	O.K.	Fringe	Large	Loosened	
49	Right			Off on $\frac{1}{3}$ of circumfer- ence	Medium	Loosened	Usual ar- rangement*
	Left	2.0	O.K.	Off on $\frac{1}{2}$ of circumfer- ence	Medium	Badly loosened	
47	Right			Off on $\frac{4}{5}$ of circumfer- ence	Medium	Loosened	Usual ar- rangement*
	Left	2.0	End of glass partly stopped	Intact	Streak about tympenic ring	None	
42	Right			Fringe	Medium	None	Usual ar- rangement*
	Left	2.0	End of glass partly stopped	Intact	Small	None	
43	Right			Fringe	Medium	Slightly loosened	Usual ar- rangement*
	Left	1.5	O.K.	Intact	None	None	

\*One shot at 15 cm. fired with the muzzle of the pistol in front of and slightly above the animal, and pointing downward along the left side at an angle of about 20° with the horizontal, just avoiding the holding apparatus with the bullet.

afforded, but I am more inclined to attribute the results obtained with this tube to the fact of its being too short to fit properly into place, thus permitting wax to pass beneath it in packing. As will be observed in Table IV, the larger tubes were used for the most part, and the results obtained with the 2 mm. tube have been discounted in considering the effectiveness of any device with which it was used on part of the animals.

Table III has been inserted in order to show the regularity with which the detonation and arrangement used caused severe injuries to the middle ear parts of

TABLE III

RECORD OF THE UNPROTECTED EAR (CONTROL EAR) OF THE ANIMALS WITH WHICH THE OTHER EAR WAS "PROTECTED"\*

GUINEA PIG NO.	CONDITION OF TYMPANIC MEMBRANE AT AUTOPSY	AMOUNT OF COAGULATE IN AND ON MUCOUS MEMBRANE OF THE MIDDLE EAR	EVIDENCE INDICATING EDEMA OF THE MUCOUS MEMBRANE
21	Off on $\frac{1}{2}$ circumference	Medium	Loosened
22	" " $\frac{3}{4}$ "	Small	
23	" " $\frac{4}{5}$ "	Small	Loosened
24	" " $\frac{1}{3}$ "	Large	
25	" " $\frac{1}{2}$ "	Large	Loosened
26	" " $\frac{1}{2}$ "	Medium	
27	" " $\frac{1}{2}$ "	Medium	Loosened
28	Only fringes left	Medium	
29	$\frac{1}{4}$ area gone	Very small	Loosened
30	$\frac{1}{2}$ area gone	Small	
31	Only fringes left	Large	Loosened
32	" " "	Large	
33	" " "	Small	Loosened
34	" " "	Large	
35	" " "	Small	None
36	Off on $\frac{1}{2}$ circumference	Large	Loosened
37	A radial slit	Only on edges of slit—no other	Loosened
38	Only fringes left	Large	Loosened
40	Whole center out	Large	Loosened
41	Off on $\frac{3}{4}$ circumference	Large	None
53	Only fringes left	Medium	Slightly loosened
54	Whole center out	Medium	Slightly loosened
55	$\frac{2}{3}$ area out	Medium	Slightly loosened
56	A wide fringe left	Large	None
57	Only a fringe	Very large	Badly loosened
58	" " "	Medium	Slightly loosened
59	" " "	Medium	Badly loosened
60	" " "	Medium	Badly loosened
61	Completely gone	Small	Slightly loosened

\*The right ear was open in all cases. For the shooting arrangement, see footnote to Table II.

the unprotected ear of the animals which had the other ear "protected." It should be recalled in studying the following tables that the gun was so placed that the protected ear received a heavier wave than the unprotected one.

## DISCUSSION

The above tabulated observations indicate the order of efficiency of the devices in reducing the intensity of the detonation wave. In discussing them I wish to add to these observations certain other factors which must be considered in selecting for military use. At this point it is well to recall that the cochleæ, when sectioned and studied, may give evidence indicating a change in this order, and very probably will help to subdivide the groups.

In the first group I would place the "Tommy" and the Mallock-Armstrong devices. Both protected the middle ears of the guinea pigs equally well. Of the two, for use in any but military life I would prefer the Mallock-Armstrong, for two reasons. In the first place, ordinary sounds are heard somewhat better; and, in the second place, if one is fitted with the proper size, the solid end of the device does not cause the irritation while wearing that the continual pressure of

TABLE IV  
RECORD OF THE "PROTECTED" EARS\*

PREVENTIVE DEVICE BEING TESTED	GUINEA PIC NO.	SMALLEST DIAMETER OF THE GLASS TUBE IN MM.	CONDITION OF WAX PACKING AFTER SHOOTING	CONDITION OF TYMPANIC MEMBRANE AT AUTOPSY	AMOUNT OF COAGULATE IN AND ON MUCOUS MEMBRANE OF THE MIDDLE EAR	EVIDENCE INDICATING EDEMA OF THE MUCOUS MEMBRANE
Scientific Ear Drum Protector "Tommy"	26	2.5	O.K.	Intact	None	
	56	3.5	O.K.	Intact	None	None
	32	2.5	?	Intact	None	None
	25	2.5	Bit of wax in tube	Intact	One area	
Mallock- Armstrong Ear Defender	21	2.5	O.K.	Intact	Two areas	
	53	3.5	O.K.	Intact	None	None
	22	2.5	Loosened	Intact	None	
Wax Cone of the Italian Navy type	33	3.5	O.K.	Intact	None	None
	34	3.5	O.K.	Intact	Very small	None
	57	3.5	O.K.	Intact	Slight	None
	60	3.5	O.K.	Intact	None	None
Cotton saturated with vaseline well worked in	61	3.5	O.K.	Intact— radial streak coagulate	Along $\frac{1}{4}$ tympanic ring	Very slightly loosened
	40	2.0 <sup>1</sup>	O.K.	Intact	None	None
	41	2.0 <sup>1</sup>	Slightly loosened	Intact	None	None
	55	3.5	O.K.	Intact	Streak about tympanic ring. Two other areas	Slightly loosened
Cotton saturated with glycerin— air was worked out as much as possible <sup>2</sup>	59	3.5	O.K.	Intact	$\frac{3}{4}$ of cir- cumference tympanic ring	None
	37	2.0 <sup>1</sup>	O.K.	Intact	None	None
	35	3.5	O.K.	Intact— Clot on outer surface	One area	Loosened
	58	3.5	O.K.	Radial slit	Edges of slit— medium elsewhere	Badly loosened
Dry cotton packed firmly	36	2.0 <sup>1</sup>	O.K.	Intact	Along $\frac{1}{8}$ tympanic ring. One other large area	
	23	2.5	O.K.	Off on $\frac{2}{5}$ circum- ference	None	Loosened
	28	3.5	O.K.	Intact	Along $\frac{1}{2}$ tympanic ring	
Elliott Perfect Ear Protector <sup>3</sup>	54	3.5	O.K.	Intact	Along all tympanic ring	Slightly loosened

TABLE IV (CONT'D)  
RECORD OF THE "PROTECTED" EARS

PREVENTIVE DEVICE BEING TESTED	GUINEA PIG NO.	SMALLEST DIAMETER OF THE GLASS TUBE IN MM.	CONDITION OF WAX PACKING AFTER SHOOTING	CONDITION OF TYMPANIC MEMBRANE AT AUTOPSY	AMOUNT OF COAGULATE IN AND ON MUCOUS MEMBRANE OF THE MIDDLE EAR	EVIDENCE INDICATING EDEMA OF THE MUCOUS MEMBRANE
Device sub- mitted to the Council by Dr. J. G. Wilson and Prof. A. Michelson	27	2.5	O.K.	Intact	Along $\frac{2}{3}$ ring. Other small areas	
	30	3.5	O.K.	Intact	3 streaks along parts of ring. Several small areas	
	31	3.5	O.K.	Hole about 3 mm. in diameter	Several small areas one large	

\*The left ear in each case; for the shooting arrangement, see the footnote to Table II.

<sup>1</sup>Controls indicate that the 2 mm. tube is too small, so the results with it must be discounted.

<sup>2</sup>A fourth animal was found at autopsy to have a thickened tympanic membrane due to an old infection, and so has not been included.

<sup>3</sup>Two more animals were tried with this device; with both the wax packing was not in good condition when examined after the shooting, and accordingly the results are not included. Both showed positive injuries.

the elastic rubber bulb of the "Tommy" does. But there is one serious objection to the Mallock-Armstrong device for army use. It has already been given in discussing the Wilson-Michelson instrument, but is so important that I will repeat it. A projectile passing alongside the head, wounding only the pinna or the outer part of the external meatus, in itself a relatively slight wound and of quite common occurrence, would shatter any hard obturator present and form secondary projectiles of the fragments, causing a much more severe wound. A further objection to the Mallock-Armstrong instrument is the difficulty in keeping dirt and mud from clogging the gauzes and membrane. There is nothing simpler to keep clean than a "Tommy" and for army use it seems to me the best of those tested.

The wax cone of the Italian Navy type comes next in order, followed closely by cotton soaked with vaseline. Because of the universal availability of cotton and vaseline I consider the degree of protection afforded by it worthy of note by those to whom the first named two mechanical devices are not accessible. The extra protection afforded by mixing the cotton with vaseline is well worth the trouble, as a comparison of this record with that of dry cotton shows.

Cotton soaked with glycerine did not protect quite so well as that saturated with vaseline; the explanation probably lies in the greater viscosity of the vaseline.

The results with dry cotton are particularly interesting in view of the unsatisfactory experience that various armies and navies have had with it in actual use. Since this was given in my previous article, it will not be repeated here. While only three animals were used, the consistency of the failure to protect is a beau-



tiful confirmation of the military experience with it. Doubtless the correct explanation is the fact of its being air-containing, as several men have urged. Cotton soaked with water was not tested; I will try it if I have occasion to run a further series with new mechanical devices. On theoretical grounds, water-soaked cotton should give protection comparable to that soaked with glycerine.

The Elliott Perfect Ear Protector and the Wilson-Michelson device rank together with dry cotton as having given the least protection to the middle ear parts of the animals used. With all three preventive measures every animal showed positive injuries, and one out of each group of three animals had a ruptured tympanic membrane.

Testing of the devices for the relative amount to which they reduce ordinary sounds has given rather contradictory results so far, due probably to the crude nature of the methods employed and doubtless in part to the individuals acting as subjects having placed the devices more or less poorly in their own ears; but this latter is a thing that will occur in the actual use of them. So I know the degree to which each excludes ordinary sounds only in a general way as yet; but I feel safe in saying that all the devices permit conversational sounds to be readily understood; loud sounds, such as commands and signal whistles, catch the attention at a considerable distance. Of the mechanical devices, the Elliott reduces ordinary sounds the least and the "Tommy" the most, the "Tommy" cutting it down more than dry cotton does, but all of the mechanical devices permit more to pass than do the wax or the soaked cotton plugs.

#### PROJECTED EXPERIMENTS

A further series of experiments is projected and will be carried out as rapidly as the time available from teaching duties will permit; it is hoped to have these completed before the cochleæ of the present series are ready to begin sectioning. Again using the rubber ear as a means of holding the preventive devices, the glass tube will be led to a tambour and the record be made on smoked paper instead of in the ear of an animal. These records are intended as a check on the relative efficiency indicated by the reaction on the guinea pigs, but could not take the place of these animal experiments, for the amount of shock necessary to cause injury will not be shown; merely the relative amount of detonation waves that pass the preventive devices. I hope by this same method, using a very delicate tambour, to obtain data on the relative amount which the various devices reduce the intensity of ordinary sounds.

#### ACKNOWLEDGMENTS

I wish to express my thanks here to Dr. Whitman for his advice on the rubber ears, and to Mr. G. C. Lubke for his careful work making the same, and to Mr. Frank O. Novy, medical student, for aid in conducting some of the experiments.

#### BIBLIOGRAPHY

For all references to literature see "War Deafness and Its Prevention—A Critical Review," published in the September, 1917, number of the JOURNAL.

## LABORATORY METHODS

### A COMPACT BOX FOR THE COLLECTION AND TRANSPORTATION OF BLOOD FOR HEMOGLOBIN ESTIMATIONS AND CELL COUNTS\*

BY STANLEY P. REIMANN, M.D., AND EDWARD P. HELLER, M.D.,  
PHILADELPHIA, PA.

THE following is a description of the box which has given much satisfaction in daily hard use in the Lankenau Hospital for several months. Bills for new blood pipettes and hemoglobinometers have fallen to zero since its introduction, while its compactness and completeness have saved much time. It is designed to accommodate nine complete counts which are collected on the wards, where the hemoglobin estimation is made immediately, and the filled pipettes are then transported to the central laboratory for counting.

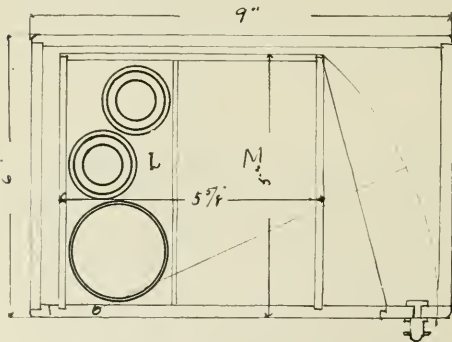


Fig. 1.—Bottom section of box.

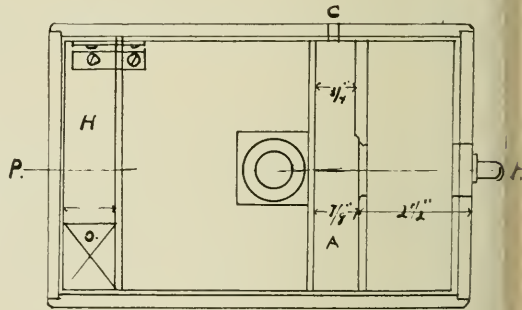


Fig. 2.—Upper compartment. *A*, space for hemoglobinometer; *H*, battery; *O*, block to hold battery in place.

The outside dimensions are 11 by 9 by 5½ inches. There is an upper compartment 8 by 4½ by 4¼ inches with a hinged lid to accommodate a Dare hemoglobinometer and cut to allow the eyepiece to project through the side of the compartment. The milled head which controls the color disc protrudes through the top of the lid, and can be moved from without, while another slot cut in the back of the compartment brings the scale into view. A small electric bulb and connections are fastened in the proper position in front of the blood and color disc; the battery is in a compartment to the side and the current is controlled by a small switch on the outside of the box immediately under the projecting eyepiece. A small piece is sawed from the top of the hard rubber movable portion of the Dare to make room for the lid to close. Ample space is left to provide small compartments for blood lancet, rubber tubing, alcohol-soaked cotton, bottles of acetic acid and saline, and bundles of horsehair and capillary

\*From the Department of Pathology of the Lankenau Hospital, Philadelphia, Pa.

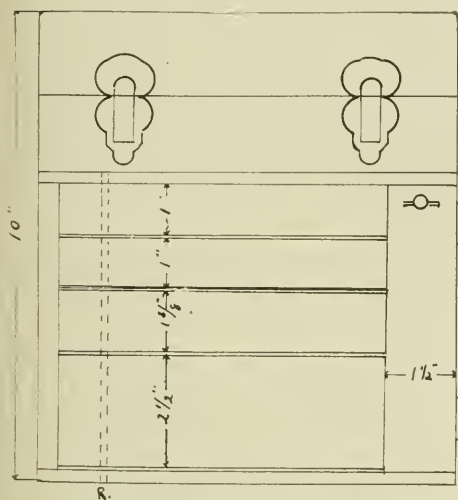


Fig. 3.—Front of box, showing four drawers and the upright which locks the drawers. The clamps for the lid are Corbin bag clamps.

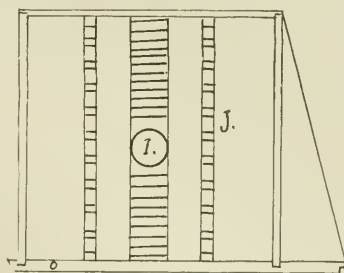


Fig. 4.—Shows a drawer for holding blood pipettes. J, coil spring; I, notched strip.

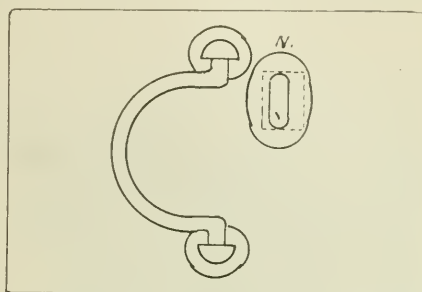


Fig. 5.—Top of box. N, opening through which the milled head of the hemoglobinometer protrudes; covered by a bulged lock escutcheon.

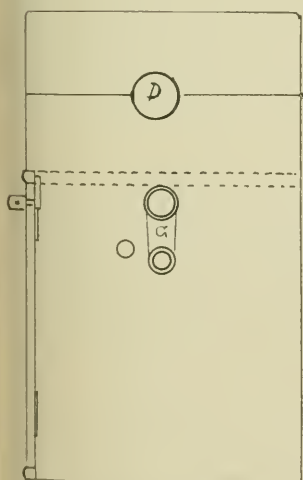


Fig. 6.—Side of box. D, hemoglobinometer eyepiece; G, switch.

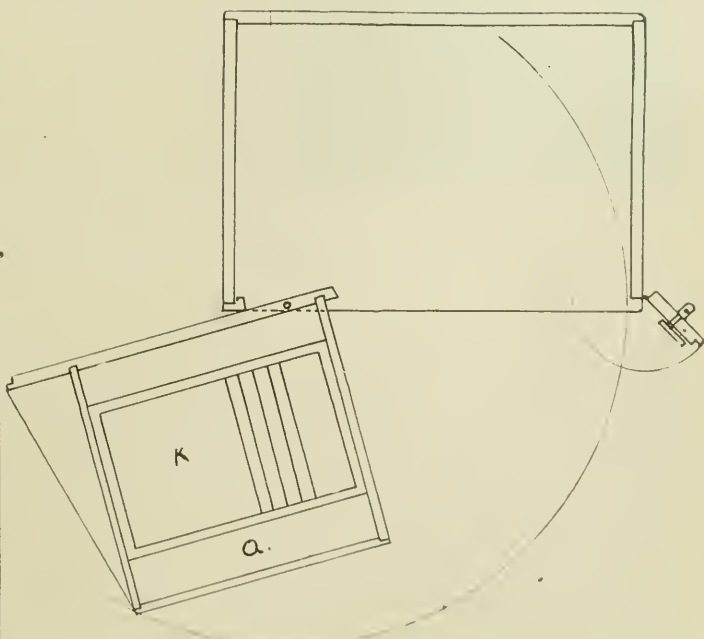


Fig. 7.—A, drawer open; O, space for hemoglobin glass slips; K, box for slides.

tubing for coagulation time estimations. The inside of the lid is painted a dull black. Comparisons of hemoglobin readings using candle light as provided for in the Dare, and the small electric light in the box have shown practically no differences.

The lower compartment, which is 8 by 6 by  $4\frac{3}{4}$  inches, inside dimensions, contains four drawers pivoted, which swing out independently or together, and are locked closed by an extension of their bottoms which are held in place by a thin hinged strip on the side of the box, and this is locked by a small turn key. In the upper drawer are small squares of gauze and cover slips. Slots are provided in the second and third drawers to hold red and white cell pipettes, and these are further steadied by a spring passing across the middle of the drawer between the turns of which the pipettes are placed. An ordinary slide box is

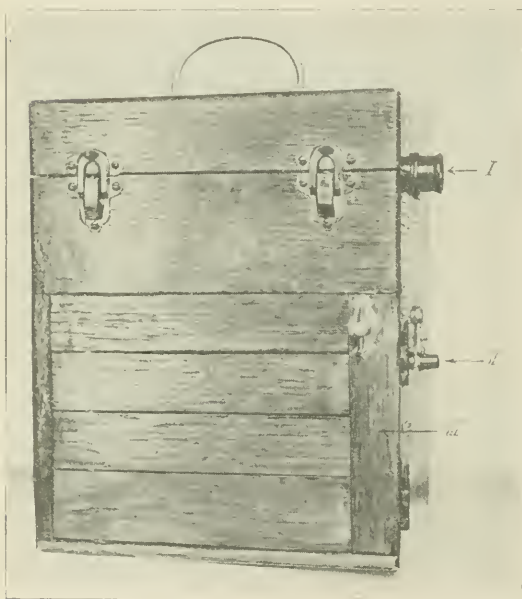


Fig. 8.—Blood box closed for transportation. I, eyepiece; II, electric switch; III, upright for locking drawers.

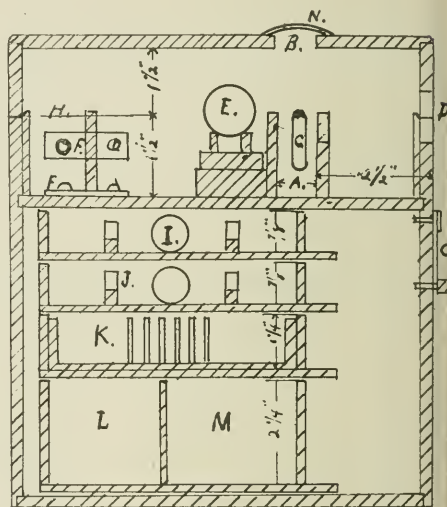


Fig. 9.—Section cut at P, Fig. 2. A, body of Dare hemoglobinometer; B, hole in lid for milled head of Dare; C, hole in back of box through which the scale can be read; D, hole in side of box for eyepiece; E, electric lamp; F, F, plates of brass with screw heads to form a circuit through lamp and switch; H, space for battery; G, switch. I, J, K, L, M, drawers to hold pipettes, glass slides, gauze, etc.

glued into the fourth drawer to hold blood smears. If they are made on cover slips, a space can be provided in the box. On either side is room for the hemoglobin glass slips and holders. The inside dimensions of the drawers are  $5\frac{1}{2}$  by  $4\frac{1}{2}$  by 1 inches. A handle on the lid provides for carrying. When filled with pipettes, etc., it weighs 10 pounds. The accompanying photographs, for which we are indebted to Dr. A. G. Miller, of the Department of Roentgenology, will show the main features.

Our thanks are expressed to Mr. E. C. Lovejoy, the house carpenter, not only for the actual workmanship, but also for many details of construction.



## AIDS TO LABORATORY EFFICIENCY

BY MILES J. BREUER, M.A., M.D., LINCOLN, NEB.

THERE is a constantly growing number of practitioners in the medical profession who realize that clinical observation ought to be supported by laboratory study. Many of these, in fact most of those who are located in smaller towns, are men who can not afford to pay a laboratory technician's salary, and who, at the same time, are too busy with their patients to spend very much time in the laboratory themselves. Even the simplest routine tests, to be of value, must be done carefully, and require time; and a man with a waiting-room full of people hardly has the patience to go through a Widal test or a gastric analysis with the same elaborateness that the internes use in a hospital laboratory.

I have always been of the opinion that even the busiest practitioner can do a considerable volume of his own laboratory work if he is so inclined, by spending a few spare hours in arranging the laboratory and apparatus so that they are convenient and efficient. By putting in a little time when he is not pressed with work, he can so arrange things that when he does have to ask a patient to wait while a test is performed, he can run through the latter with a minimum expenditure of time and energy. The main essential is to eliminate unessential motions and operations, and it is surprising how much one's time in the laboratory can be cut down, by attention to a few simple things. Especially since it is now possible to purchase reagents, media, and stains ready made-up or nearly so, is it desirable for every physician to do as much of his own laboratory work as possible, in preference to not having it done at all.

Below are described a number of methods and pieces of apparatus which have enabled me to do a great deal of clinical laboratory work, although I have only a limited amount of time to spend in the laboratory. Some of the ideas are original, some are not; the sources of the latter are mostly forgotten and not recorded. Naturally, these things will be of interest rather to the practitioner than to the man who is at home in the laboratory.

In order to run through a test with rapidity and certainty, it is necessary to have it well in mind. The next best thing, when this is not practicable, is to have a card index of the tests in common use. The preparation of such an index is not as much of a task as might at first thought seem, for when the test is being studied over for the first time while learning to do it, it takes but little additional time to outline or abstract it on a card. Later when the work is being done, it is only necessary to take the card from its box, which should be kept in the laboratory, and keep it in front of oneself while working.

Take the matter of making a Gram stain, or a stain for tubercle bacilli; the average physician is apt to consider the procedure rather tedious; and it is tedious, if he takes the slide in a pair of forceps, holds it over an open flame, and drops the stain on it out of a bottle each time. For the tubercle method I have provided an iron plate, which I heat over a Bunsen burner, hot enough to make a drop of water sizzle; on this I lay the slide, removing the burner, and

covering the slide with carbol-fuchsin, leaving it there for five minutes, during which time I can be attending to something else. For the rest of the process, and for the Gram stain and other special stains, I use Coplin jars filled with the stain. For destaining, I have Coplin jars filled with the necessary alcohol. With these, staining is merely a matter of transferring the slide from one jar to another, and while it is in the jar, other work can be attended to. In case of most of the stains, it makes little difference if the slide is left in the stain longer than the time prescribed in the textbook. In place of the Coplin jars, ordinary wide-mouthed bottles, deep enough to cover a slide, may be used.

Reaching up on a shelf, taking down a bottle, removing the stopper, and pouring out the reagent, consumes an appreciable amount of time, which be-

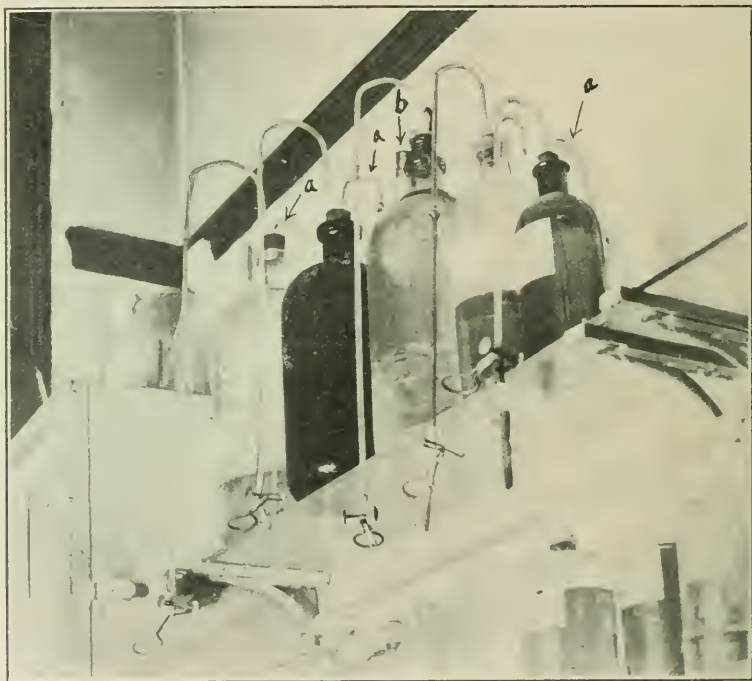


Fig. 1.—Siphon bottles.

comes considerable when frequently repeated. The installation of siphon delivery bottles for the more common reagents so that merely the pressure on a pinchcock suffices to release the flow, is easy and needs no further explanation than the illustration (Fig. 1). At *a* is a tube plugged with cotton, to allow ingress of air to replace the liquid that runs out. I use this method for handling the following: distilled water and physiologic salt solution, both kept sterile; *N* 10 sodium hydroxide, which is protected by a wash bottle containing caustic soda sticks *b*, to remove the carbon dioxide from the entering air and avoid weakening the solution; Benedict's solution, and alcohol. Tenth-normal sodium hydroxide and hydrochloric acid are also kept in burettes fastened to the wall, ready for instant use. The top of the burette should be stoppered with a rubber

stopper, which is removed when the burette is used. The sodium hydroxide burette should have a rubber delivery tube and pinchcock instead of a glass stopcock, as the alkali causes the glass to stick. It would be desirable to keep nitric acid in such a siphon delivery bottle, as it is very frequently used, but

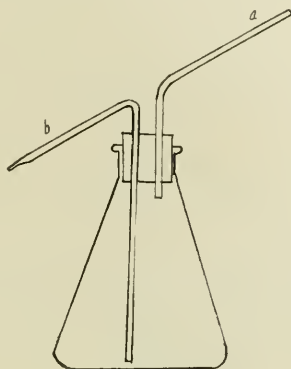


Fig. 2.—Wash bottle.

it is a little too energetic to be handled this way, as there is danger of its being scattered about.

Another point in the handling of reagents, is the use of wash bottles for distilled water, physiologic salt solution, and alcohol. Very frequently during the

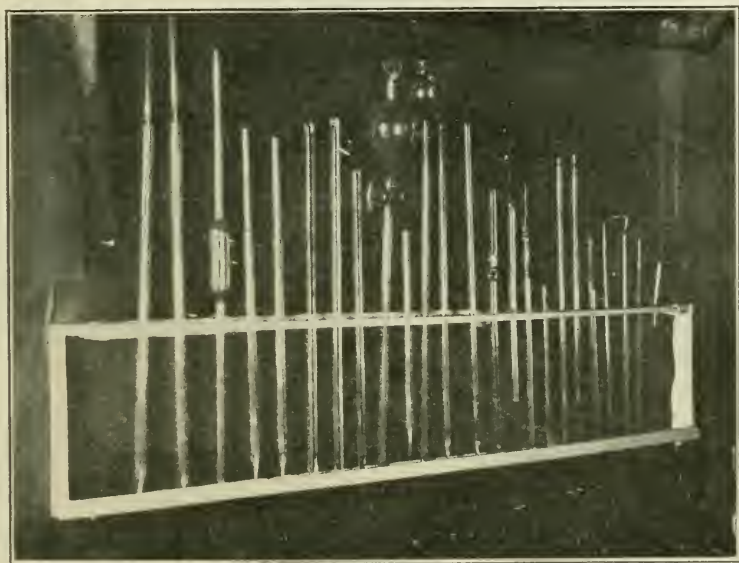


Fig. 3. —Pipette rack.

course of tests it is necessary to use small amounts of these substances. If they are kept in wash bottles of the form shown in Fig. 2, blowing into tube *a* will deliver a small quantity, or inclining the bottle so that the fluid runs out of *a* will deliver a larger quantity.

In most laboratories that I have seen, pipettes, glass rods, platinum needles and loops are kept in a drawer; when one is needed, it is necessary to open the drawer and rake among its contents to find what is wanted. I have adopted the pipette rack shown in Fig. 3, made of thin pieces of wood fastened to the wall over the work table; this keeps all the rods and pipettes in plain sight and instantly available. It consists of an upper strip with holes through which the pipettes are placed, and a lower strip with hemispherical depressions made with a twist drill. At the bottom of each of the latter is a small hole entirely through the wood, to drain out the water, when pipettes are placed wet in the rack.

It is possible to save much lost motion by attention to small matters about the use of a microscope. The light is important. I never use daylight, but have

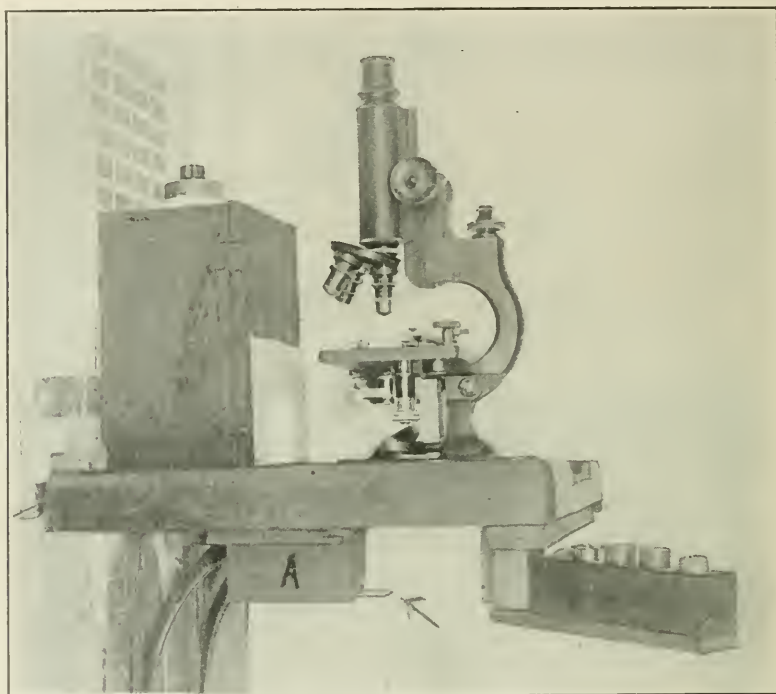


Fig. 4.—Microscopic shelf.

a 100-watt nitrogen Mazda lamp and a blue filter, which will give correct color rendering. This gives a constant standard illumination for comparison, and is instantly available, day or night, with no other arranging or manipulation than the mere turning of a switch. Many laboratories have the light under the table, coming up through the base of the microscope, whose mirror is swung out of the way. This method is good if it is possible to interpose a convex lens of proper focal length; otherwise definition suffers. The condenser of the microscope is made to handle parallel rays. If the light comes from a source closer than four feet away, it is necessary to use some method of rendering the rays parallel; otherwise the best results can not be obtained.

I have a special shelf fastened to the wall for microscopic work. The



various extra oculars, and bottle of immersion oil, that come set in a block in the microscope box, were pivoted with their block on the under side of the shelf, so that they can be swung out when needed. An arrangement has also been provided which always keeps a clean slide within easy reach. This is shown at *A* in Fig. 4, with an arrow pointing to a partially delivered slide. Fig. 5 shows the details of construction. In Fig. 4, above the box containing the light, is shown a chart of brightly-colored squares for the purpose of resting the eyes when fatigued by prolonged microscopic examination.

The cleaning of hanging-drop slides is a tedious operation for the busy man, especially when they are contaminated with pathogenic organisms. Much time can be saved by the arrangement illustrated in Fig. 6. A cylinder is used, made from a short section of half-inch glass tubing, ground flat on both ends by an optician. The shorter the section of the tubing, the better the definition obtainable, as the drop is then closer to the focus of the rays from the condenser. The cover-glass with the hanging drop is set on the cylinder as shown, and nothing becomes soiled or contaminated except the cover-glass, which can be taken

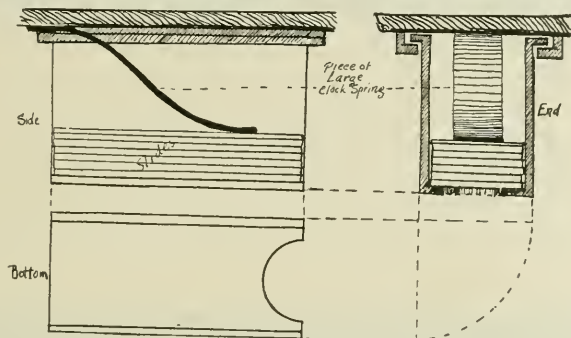


Fig. 5.—Container for slides.

off after the examination is over, and dropped into a jar of sulphuric acid and potassium dichromate mixture, which is always kept standing in the laboratory to receive slides and other small pieces of apparatus requiring cleaning. By using such cylinders, two or three hanging drops may be kept together on the same slide, which is desirable, for instance, when the agglutination tests for typhoid and the two paratyphoids are being done.

An ordinary suction pump, operated by water pressure, such as is screwed to the water faucet, is a great time saver about the laboratory. Pressure tubing (such as is used on stethoscopes) is used for the connections, and a large bottle is interposed in the suction tube by means of two bent glass tubes in a rubber stopper; the purpose of this is to catch fluids that are being aspirated, as their passage through the pump will interfere with its action. This pump can be used, for instance, for cleaning blood counting pipettes. The pipette is slipped into the connecting tube, and dipped for a moment into water, alcohol, and ether, the suction drawing through enough of the fluid to clean the pipette, and the entire operation not requiring over one minute. Pipettes for serum work, and small measuring pipettes can be cleaned in the same manner. For separat-

ing serum from corpuscles, and separating other liquids, decanting, and to replace siphonage, this apparatus saves much time and work. When it is desired to keep the separated fluid, a small wash bottle can be interposed on the connection to catch it. For aeration methods, the pump is indispensable. I have made this pump do duty in gastric aspiration, and in the office operating room in mouth work, to get rid of saliva.

Fig. 7 illustrates a form of pipette that I have found very convenient for serum work and work with vaccines, especially in connection with the pump

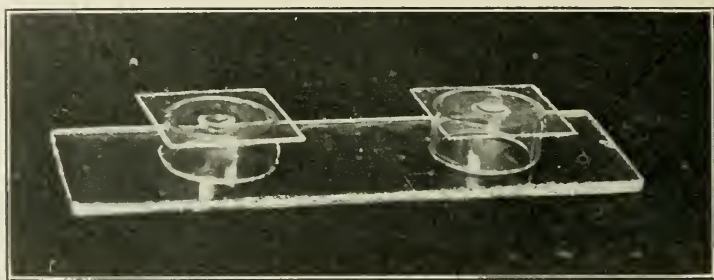


Fig. 6.—Hanging-drop arrangement.

mentioned above. These pipettes are easy to make, and can be made over a Bunsen burner from ordinary glass tubing after a little practice. They are used in separating serum, decanting in washing corpuscles, filling ampules, and the like.

When it is necessary to take a specimen for a blood count and carry it some distance, as for instance from the patient's home to the laboratory, the transportation of the filled pipette without affecting the accuracy of the test

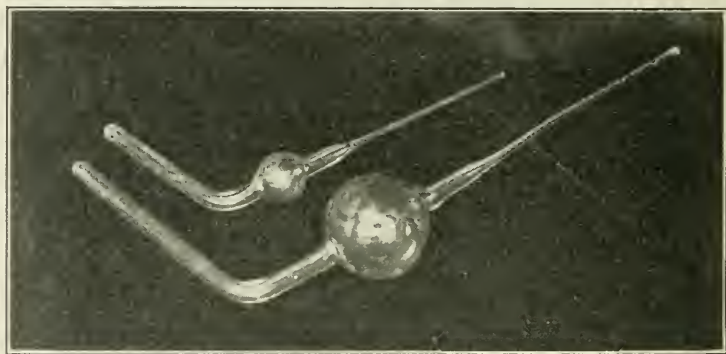


Fig. 7.—Serum pipettes.

has always been a problem. Obviously, the ideal thing is to have a portable microscope, but this is out of the reach of many. I have found that a short piece of heavy inner tube from an automobile tire, forming a heavy rubber band, is an excellent method, the band being placed around the pipette so that pressure is exerted on both orifices.

Another little thing that offers some difficulty for the average practitioner doing his own laboratory work, is the dilution of serum for Widal work. I am

of the belief that this dilution should be made with a fair amount of accuracy; if one merely slaps down a drop of serum and a few drops of water, the test might as well not be done at all. When a large amount of serum is available so that it can be measured in drops or tenths of a cubic centimeter, the dilution is not difficult. The effort should be made to procure a large enough amount of blood; but the fact remains that this is not always done, nor is it always even possible or practical. When only a small quantity of serum is available, it may be measured thus: draw out a glass tube to a long capillary, as much as eighteen inches if possible. Cut off one end at a portion where the bore begins to be uniform; retain the other end as a mouthpiece for blowing out the serum. The serum is allowed to flow in by capillary attraction, for a certain length of the tube measured with a ruler, then the proper quantity of salt solution for dilution, measured by length, taking care to use only that part of the capillary which is of uniform diameter.

None of the apparatus mentioned above is out of reach of the average practitioner, even of the young man starting in practice, either in respect to expense, or skill required in its construction. By conducting efficiently those laboratory operations which he owes as a duty to his patients and himself, he can make the laboratory a pleasant part of his work rather than irksome drudgery.

# *The Journal of Laboratory and Clinical Medicine*

VOL. III.

JANUARY, 1918

No. 4

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.

Ann Arbor, Mich..

## ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D.	-	-	ST. LOUIS
HANS ZINSSER, M.D.	-	-	NEW YORK
PAUL G. WOOLLEY, M.D.	-	-	CINCINNATI
FREDERICK P. GAY, M.D.	-	-	BERKELEY, CAL.
J. J. R. MACLEOD, M.B.	-	-	CLEVELAND
ROY G. PEARCE, M.D.	-	-	CLEVELAND
ROGER S. MORRIS, M.D.	-	-	CINCINNATI
GERALD B. WEBB, M.D.	-	-	COLORADO SPRINGS
E. E. SOUTHARD, M.D.	-	-	BOSTON

Contents of this Journal Copyright, 1918, by The C. V. Mosby Company—All Rights Reserved  
Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

## EDITORIALS

### *Measles and Pneumonia in Our Camps*

MEASLES and pneumonia are so closely associated in military camps that it is well to consider them together. Measles is one of the most infectious diseases and its restriction in camps seems well nigh impossible except under exceptional conditions when the soldiers can be scattered over large areas. This requires so much housing in the form of tents or barracks, and interferes so greatly with drill and other military activities, that measles is justly greatly dreaded.

The soldier who has had this disease in childhood is fortunate, and a command made up of immunes is doubly so. One attack seems to give life-long protection. It is true that occasionally a soldier, who is sure he has had measles, develops this disease; but it is more than probable that the former attack was due to some other eruptive disease. Organizations recruited from sparsely settled districts are more prone to this infection than those from urban or more thickly settled communities, on account of the larger number of susceptible individuals.

Measles, among our people at least, is seldom fatal so long as it remains



uncomplicated and without more serious sequelæ. This is so generally true that it has been seriously suggested that all susceptibles should be exposed to it in order to secure immunity, but until the complications and sequelæ can be more certainly controlled, this procedure can not be recommended. The control of epidemics of measles is more difficult because it is transmissible before it is recognizable in the individual. This, however, should not deter medical officers from the daily inspection of all men under their charge, and especially of all recent additions to their commands.

On the first evidence of the disease, the soldier should be sent to the hospital and his tentmates should be quarantined for 14 days. This time is believed to cover the period of incubation. The contacts may be drilled in a squad by themselves, but should not be allowed to mingle with others. Their drill master and attendants should be selected from those known to be immune.

A soldier sick with measles should be immediately sent to the hospital, with his clothing and blankets. On arrival at the hospital, the clothing and blankets should be sterilized by steam, rolled in paper, or placed in a paper bag, labeled and put away awaiting the recovery of their owner.

In the hospitals, patients with measles should not be crowded more than is absolutely necessary. However, when the bed capacity is doubled by the number of patients, as has happened in more than one base hospital recently, overcrowding seems unavoidable unless additional cot space can be secured by the immediate erection of hospital tents. When the beds must be brought within four feet of one another, an improvised clotheshorse carrying a sheet kept moist with a 5 per cent solution of carbolic acid, or a 1:1000 solution of bichloride of mercury, should be placed, as a screen, between the cots. This may seem wholly unnecessary, and it might be asked why it is necessary to try to protect one measles patient against another. The purpose of this recommendation is not to prevent the spread of the measles; but some of these patients, experience indicates that many of them, are carriers of virulent pneumococci and streptococci, and the purpose of this precaution is to prevent the dispersion of these virulent organisms. The sputum cups should be burned and the floors should be kept moist and dry sweeping should not be permitted. The patients should be kept warm and given hot drinks. The plates, knives, and forks should be boiled. Drinking cups should not be exchanged, but each patient should have his own. Paper napkins should be used and should be burned with the sputum.

A mistake made in many of our camps is the too early return to duty of soldiers recovering from measles. They should be placed in convalescent hospitals and kept at rest for a period of ten to fourteen days before returning to duty. While uncomplicated measles seldom or never kills, it lowers the resistance to infection with pneumococci and streptococci. Too often it has happened that a soldier returned to duty after recovery from measles is sent back to the hospital within two or three weeks or earlier with pneumonia. On the other hand, in those camps in which the convalescents from measles have had proper care, the number of cases of pneumonia is relatively low. It should be impressed upon every medical officer that a convalescent from measles is especially susceptible to pneumococcic and streptococcic infection, and in order to protect him

from these infections, he should have proper and prolonged professional supervision.

When a soldier recovers from measles and leaves his bed in the hospital, the bedding should be sterilized by steam before it is occupied by another patient. The absence of any provision for this procedure is, up to the present time, a striking feature of most of our base hospitals. Sterilizers, we are told, have been ordered, but up to the early part of December we found no base hospitals fitted with facilities for sterilizing mattresses and blankets. It is claimed by many that exposure for a few hours to the air—we will not say to sunlight because this is by no means always in evidence—is sufficient to disinfect bedding which has been occupied by patients with measles and pneumonia, but some are still skeptical on this point.

That overcrowding has been a factor in the spread of respiratory diseases in our camps seems quite evident. The transmission of these diseases is in inverse ratio to the distance between individuals. With men sufficiently scattered, even measles—as contagious as it is—is not transmissible. The Surgeon-General, with his extensive experience with septic pneumonia in the Canal Zone and in South Africa, has recognized the danger from the respiratory diseases and has insisted from the first that every man in barracks or tents should have a minimum of 50 square feet of floor space. With nine men in a tent 16x16, the space is reduced to less than 29 square feet and in some camps the number of men in such tents was as great as 12. Soldiers have been overcrowded in tents, barracks, and even in hospitals.

The viruses of the respiratory diseases are transmitted for the most part, at least, through the spray present in coughing, sneezing, and rapid talking. It is true that a virulent pneumococcus has been found in dust, and this can not be omitted from the list of distributing agencies. Some of our camps have been very dusty, and how greatly this has contributed to the prevalence of the respiratory diseases can not be accurately determined.

Medical officers should be awake to the danger of overcrowding and should do what they can to avoid it. Line officers have certain valid reasons for preferring compact organizations. It favors administrative purposes. However, there should be no unreasonable conflict on this point among intelligent men, all of whom must recognize the fact that the strength of an army lies in its effective men and is in inverse ratio to those on sick report and in hospital. The medical officer is supreme in the hospital, and still some wards have been overcrowded while others are vacant. Because a ward is intended, in the construction of the hospital for surgical cases, is no reason why it should remain vacant for weeks while the pneumonia wards are carrying twice the number of patients they were built for. If the sterilization of bedding is secured by a few hours' exposure to out-of-door air, a ward should be safe after the removal of all the beds and a few days' exposure with open windows and doors.

In our base hospitals in this country, with no wounded, the greater part of the space, which means the greater number of the wards, should be used for medical cases and especially for respiratory diseases.

It should not be forgotten that overcrowding in sleeping quarters is deter-

mined, to some extent, by the position of the cots with reference to one another. So far as the spread of respiratory diseases is concerned, men may be overcrowded in the open air. It has not been unusual to find men sleeping in cots so arranged that four heads are brought close together. The medical officer should see to the arrangement of cots in tents and barracks. On account of the shortage of blankets and the absence of heating facilities, it is not rare to find two cots brought close together so that the same blankets may cover two men.

It seems to have been assumed by those who had supervision of the housing of our soldiers, that camps and cantonments in the southern states did not need to be warm, and has probably been a contributing factor in the greater prevalence of pneumonia in the southern camps compared with those located in the north. Moreover, it is an old and well certified medical observation that pneumonia is not only more common, but more fatal, in the south than in the northern states. While the average winter temperature in Michigan is lower than that of South Carolina, one may suffer from the cold quite severely in the latter state. The same general idea seems to have prevailed among those whose duty it was to provide winter clothing. This was distributed last in the southern camps, and as late as early December many of the soldiers at Fort Worth, Texas, were not provided with woolen clothing. By one who has experienced the chilling effects of a Texas norther, this defect will be appreciated.

Another mistake in the construction department was to leave the base hospital for the last building to be erected. At Fort Worth, early in December, the base hospital was without running water and sewers, and all bed pans had to be carried a long distance—in some wards as far as a quarter of a mile. There were absolutely no bathing facilities in the base hospital. At Fort Sill, the base hospital was in process of construction, and some of the wards, partially built, were crowded with patients. There were no trained nurses, and the soft coal used in the small stoves was tracked over the floor and the dust was deposited on sheets and pillow cases. In camps and cantonments in this country, the base hospital should be completed, and furnished with every facility, before troops are moved in.

The excellence of the barracks of the aviation corps, and the relative freedom from disease among those occupying them is shown when comparison is made between them and the division housing. It is said that the former were constructed by the aviation corps, not by the quartermaster's department. The construction cost more in money, but, so far, has cost much less in sickness and deaths.

A great sin has been committed in sending troops from a camp known to be badly infected, to one relatively free from infection, without proper precaution. At Camp Wheeler, Macon, Georgia, and at Camp Beauregard, Alexandria, Louisiana, the statement is made that their infection came from Camp Pike, Little Rock, Arkansas. At the last mentioned place, the claim is made that their infection came from Camp Funston, Fort Riley, Kansas. Indeed, the charge is openly made that one camp intentionally emptied its hospital into another camp. While this undoubtedly is a gross exaggeration, there is some truth in these statements. Men have arrived at their destination actually ill with measles,

pneumonia, or meningitis, and have been sent directly from the train to the hospital. Such procedure would not be permitted by civil health authorities. Moreover, this is not the worst of it. Men coming from an infected camp have been immediately distributed among the different organizations at the place of arrival. In this way, the infection has been planted and scattered widely in a most fertile soil. Under no condition, except under the stress of actual warfare, should troops be sent from one camp to another without isolation for a period under medical supervision both before departure and after arrival. This precaution should be taken on all transfers of troops from one camp to another occupied camp. When the movement is from an infected camp, extra precautions should be taken. New arrivals, whether they come from another camp, or from recruiting stations, or directly from their homes, should be quartered apart and inspected individually and daily before distribution among organizations. The transfers of troops should be under the supervision of medical officers, and these should report to the medical officers at the place of destination all infections which have appeared among the arrivals or to which they have been exposed. These precautions might not be necessary among well-seasoned troops who have been in the field for months and possibly for years, but with raw soldiers they are absolutely necessary if infection is to be reduced to a minimum. Within a few months, we should have a constant stream of troops moving from their homes through one camp and another to points of embarkation across the ocean to the battlefields of Europe. To protect these millions from infection, or to reduce infection among them to the minimum, will require the constant attention and care of our most experienced epidemiologists. We can not hope to avoid loss from infection altogether, but we must reduce it so far as possible. We must pay a penalty for the unprepared state into which we allowed ourselves to fall, notwithstanding the warning which came to us so plainly more than three years ago. The seasoned soldier learns to take care of himself, and, if we are correctly informed, the infectious diseases are infrequent among the French and English veterans, but they have had long training. At best, we are to pay dearly for our apathy.

When the new select men are called, each should come to camp with a card from the health officer of his home, giving a list of the communicable diseases observed in that locality during the past month. These men should be housed in groups of not more than 30, and preferably in much smaller groups, kept in quarantine with daily medical inspection for at least two weeks. Their civilian clothes should be disinfected and sent home. The men should have thorough cleaning in supervised baths and under the care of an officer. They should be clothed in proper uniforms and supplied with an extra suit of underclothing. During the period of detention, the men should receive their vaccines and undergo all necessary special medical examinations. Each group, or squad, should be drilled or exercised by itself. At the end of two weeks, groups which have remained free from infection, might be brought together—not more than two groups at first. After shorter intervals, companies may be formed, and finally regiments, but for two months the groups should not be larger than companies. After this, regiments and larger masses may be organized. In the



primary training camps, the arrangement should be such that each company could be isolated from every other part of the camp at short notice. Medical officers especially skilled in the recognition and effective management of communicable diseases, should daily examine every man, stripped and while in his bath. Daily bathing should be a routine exercise. Medical officers, during this preliminary period, should live with the men, eat at the same mess, sleep in the same quarters, and should, at the end of that time, have some voice in the selection of noncommissioned officers. Besides, success in this work should be given weight in fixing the rank of medical officers. All the camps and cantonments now in this country should be maintained at least as long as the war lasts. This is recommended in order that overcrowding may not again be necessary, and, if the number now existent is not enough to prevent overcrowding, more camps and cantonments should be provided. The barracks and base hospitals now in existence should be painted and kept in repair. This should include the water supply, sewerage, baths, etc. Let us proceed as though we expect the war to continue indefinitely. When it does stop, these camps and cantonments may be used for the universal military training for which it is to be hoped we will be wise enough to provide. Some of the base hospitals would be excellent for tuberculosis sanatoriums, if kept in repair. There is nothing more demoralizing to camp sanitation than the idea that the camp is soon to be abandoned.

With the restriction of the death rate from tuberculosis, pneumonia is fast winning the rank of "Captain of the hosts of death." There is a general impression that it is most prevalent among children and the aged, but Cole states that more than half the cases fall between 20 and 50 years—the period of greatest activity. It was the most serious disease met with by the Surgeon-General in the Canal Zone and he was called to South Africa to advise concerning its restriction among the miners in the Rand region. With the exception of dysentery and typhoid fever, pneumonia caused more deaths among the soldiers of the Civil War than any other disease, and with the elimination of typhoid fever, pneumonia in all probability will claim more deaths in our army than any other disease. Experience shows that it is especially prevalent among recruits. It levies a heavy tribute upon those who are passing through the transition from home life to that of the soldier. Many fall its victims in the process of being adapted to the altered conditions of life. Especially is this true when the period of this transition is accompanied by exposure to unusual cold and wet. The fact of this greater prevalence and fatality among the inhabitants of our southern states, has already been mentioned, as has the special susceptibility of those convalescent from measles.

It is customary to speak of lobar and bronchopneumonia. This distinction is useful from the standpoint of the pathologist, but how useful it is from an etiologic or epidemiologic viewpoint remains to be seen. Both forms may be primary and both may follow measles. It is generally believed that lobar pneumonia is more frequently primary, while bronchopneumonia is the form most likely to follow measles. However, up to the present time there are no convincing statistics on this point. It is highly desirable that accurate observations should be made and recorded in our base hospitals as to the relation of the two

forms of pneumonia to measles. The records should show in each case as to whether it was lobar or bronchopneumonia, but what is of more importance, whether it was primary or post measles pneumonia. With accurate observations on these points, properly recorded, we should soon have information of great value in an epidemiologic way, and this is what we greatly need in our efforts to reduce the incidence of these diseases.

Cole defines lobar pneumonia as follows: "Acute lobar pneumonia is an acute infectious disease, the characteristic feature of which is a uniformly diffuse exudative inflammation of entire portions of one or more lobes of the lung. It has long been a question of dispute, however, whether the definition of the disease shall be based on the pathologic, etiologic, or clinical features. In our opinion, so far as prevention and cure of the disease are concerned, it is of the greatest importance that the chief stress should be laid on the etiologic agent. Lobar inflammation of the lung may undoubtedly be caused by a number of different bacteria. The vast majority of the lesions, however, are caused by varieties of *diplococcus pneumoniae*."

The bacteriology of bronchopneumonia has not been so completely studied, but it is safe to say that the varieties of bacteria found in the exudate are more numerous, and, while the pneumococcus may be the sole causative agent, streptococci are more frequently present than in lobar pneumonia and may be the chief, if not the sole, causative agent. Careful observation and records in the laboratories of our base hospitals should give us most desirable information concerning the bacteriology of both forms of pneumonia, and there is opportunity in each of these laboratories to make useful contributions to the etiology and epidemiology of pneumonia.

The pneumococcus was first found by Pasteur in the saliva in a case of rabies, and about the same time by former Surgeon-General Sternberg in the normal saliva. The fact that many people in health carry these organisms in their saliva more or less constantly, led to the belief that pneumonia results from the lowered resistance of the individual induced by unusual exposure to wet and cold, an attack of measles, etc., and that isolation and disinfection of sputum was unnecessary. Under this attitude toward the disease, it has not decreased and there is strong evidence that it has increased. The census of 1900 made it responsible for more than 10 per cent of all deaths.

The painstaking investigations of Cole and his associates at the hospital of the Rockefeller Institute have shown that pneumococci may be differentiated into several types and subtypes. This work is still incomplete, but it has progressed far enough to throw much light on the etiology of lobar pneumonia, and to justify us in taking quite a different view concerning the methods that should be followed in attempts to limit the spread of the disease. In a study of the mouth secretion of 297 individuals in health and without history of recent contact with a case of lobar pneumonia, 181 were found to be free from any form of the pneumococcus, while 161 showed some form of this organism. This indicates that more than half of normal individuals, without recent exposure, are free from pneumococci. In the 161, the types were distributed as follows:

Type I — 0.8 per cent	Type IIb— 5.8 per cent
Type II — 0.0 per cent	Type IIx—11.6 per cent
Type IIa— 0.8 per cent	Type III —28.1 per cent
Type IV —59.9 per cent	

In 458 cases of lobar pneumonia, the distribution of the types was quite in contrast with the above as shown by the following:

Type I —33.3 per cent	Type IIb— 0.9 per cent
Type II —29.3 per cent	Type IIx— 2.0 per cent
Type IIa— 1.3 per cent	Type III —13.0 per cent
Type IV —20.3 per cent	

"Comparison of these two tables shows that the pneumococci most commonly found in the mouth secretions of normal individuals give rise to a minority of the cases of lobar pneumonia. The disease produced by these organisms, with the exception of Type III, is less severe in character, indicating a lower grade of pathogenicity of those types for man. On the other hand, Types I and II cause a majority of cases of lobar pneumonia, are of high virulence for human beings and are seldom or never found in the mouth secretions of normal individuals who have not been in intimate association with cases of lobar pneumonia. This seems to indicate that lobar pneumonia due to Types I and II does not rise from infection with a pneumococcus which is habitually carried in the mouth, but that infection with these organisms occurs from without."

Men trained by Cole and familiar with his methods of differentiating the types of pneumococci have been placed in the laboratory of each camp in which pneumonia prevails, and we may expect much new information along this line. Cole has developed a serum which apparently gives good results in those pneumonias due to Type I. This serum is being used in cases shown to be due to this type. However, we are at present more deeply concerned with the prevention than the treatment of these diseases, and in our efforts at prevention we are proceeding on the assumption that pneumonia is a transmissible disease, and that the causative agent at least in a large percentage of cases comes from without. In doing this the probability that susceptibility may be increased by unaccustomed and untoward conditions of life must not be overlooked. Indeed, the truth of this is indicated quite conclusively by observations extending back for centuries and recognized in many parts of the world.

We may laud the advantages of free ventilation, out-of-door living and sleeping, the hardening and bracing effects of cold weather, cold bathing, etc., as much as we please, but we must admit that when men in masses suddenly change their residence from warm, unventilated or poorly ventilated homes, to the life of the tent and the open barrack, they need warmer clothing and heavier bedding, and if these are not furnished, pneumonia takes heavy toll from their ranks. Furthermore, it seems true that the more radical and sudden this change, the greater is the toll. The Michigan lad may have his snow bath or his plunge through the broken ice, and quickly get back into his heavy woollens with impunity, but when the South Carolina youth indulges in such an experience and goes back into his cottons, insurance on his life ceases to be a good risk. The French have found it absolutely necessary to provide warmer quarters and heavier

clothing in the winter for their African contingents than is needed by the native Frenchmen.

Hookworm infection among the southern select men has been suggested as an explanation of the lesser resistance they show toward respiratory diseases. It may be a fact, but so far this has not been demonstrated. These facts long known to medical men seem to have been without the ken of those who provided or failed to provide quarters and clothing for the recent recruits in the southern camps. It is for the future to determine whether the Cracker from Georgia will make as hardy a soldier as the Wolverine from Michigan. Both bore many privations in the Civil War and proved themselves worthy antagonists.

To the suggestion that lack of suitable clothing has been a factor in the prevalence of pneumonia in the camps, the reply is made that warm garments in sufficiency are now in the camps or on the way to them. To the man who died of pneumonia in the base hospital in Fort Worth last month, last week, or yesterday, this reply is of small comfort. However, this is no time to look backward, but it is the time to look forward and to see that mistakes are not continued. It is the duty of the medical officer to protest repeatedly and vigorously, if necessary, through official channels, if those under his care suffer from insufficient clothing or bedding. It is also his duty to see not only that they have proper clothing, but that they wear it.

That overcrowding favors the dissemination of pneumonia can not be questioned. It multiplies the number of those within the infection range, increases the contacts and makes segregation more difficult.

On entering an amusement or assembly hall in one of our cantonments and hearing many coughing and filling the air with germ-laden spray, one can not easily dismiss the impression that these places serve as distributors of the respiratory infections. A like impression has evidently been made upon those in authority, for in several camps places of assembly have been closed and similar extra cantonment picture shows have been barred to the soldier. This procedure is regrettable but nevertheless commendable. In the process of making soldiers, some pleasures must be curtailed.

The soldier sick with pneumonia, either primary or post measles, should be immediately carried to the base hospital, and, so far as protective measures are concerned, should be treated much the same as has been recommended for those with measles. Overcrowding in hospital and the screens between beds should be remembered, the one to be avoided and the other to be provided. An individual with Type I of the pneumococcus may distribute his more fatal infection to a neighbor originally infected with the less virulent Type IV, or a streptococcus infection may be engrafted on a pneumococcus growth. The fact that empyema frequently develops in both lobar pneumonia and bronchopneumonia suggests that the streptococcus is present, either as a primary or secondary factor. Moreover, in several hospitals pneumonia has developed in physicians and other attendants. Whether patients with pneumonia do better in a cold or in a warm, moist atmosphere seems to be an undecided question. While this is being determined, it is quite certain that whatever the temperature of the inhaled air may be, the body should be protected from chill. Among those most



experienced in the treatment of pneumonia, the value of digitalis seems to be conceded, but there are differences in opinion as to the extent to which this drug should be administered, as to the indications for pushing or decreasing its administration, and as to the preparation of this drug most suitable.

Inspection of our camps and their hospitals leaves one with the impression that so far as their medical personnel is concerned, the greatest need is for men skilled in the early recognition and the handling of the acute respiratory diseases. Valuable as improvements in the determination of the types of the pneumococcus and the development of curative serums are, the prevention of infection and the limitation of its spread are far more important. The daily mail of the Surgeon-General's office brings suggestions, recipes and offers to sell to the Government "sure cures" for pneumonia. We can leave the treatment of this disease to the wisely selected medical officers in the base hospitals. What is needed is knowledge whereby we can prevent this disease. However, the mere possession of knowledge is of no value unless it be accompanied by the means necessary for its application. Insufficient clothing, overcrowding in tents, barracks and hospitals, and lack of heat in the houses, have been potent factors in the development of the pneumonia among our soldiers, and for these deficiencies the medical corps is not responsible. This is not intended as a criticism on anyone, nor does it imply that the medical corps would have done better if it had been given authority in all these matters. It does mean that in the making of an army, the health of the soldier is of first importance and this can be secured only by the intelligent cooperation of all those who are responsible for the conditions under which he lives.

The Surgeon-General has asked:

- (1) That deficiencies in clothing be made good.
- (2) That additional tentage and housing be provided, sufficient to give each soldier at least 50 square feet of floor space.
- (3) That a detention camp be established with each division, and in which all arrivals may be kept under medical observation for such time as may be necessary.
- (4) That a quarantine camp be established in connection with each division and in which contacts, suspects, and carriers can be isolated and observed.
- (5) That, where necessary, tents or other housing should be provided for convalescents.
- (6) That unfinished hospitals be hastened to completion, including water, sewers and baths.
- (7) Additional nurses, when necessary.
- (8) The enlargement and better equipment of the base hospital laboratories where desirable.
- (9) The selection and detail of medical officers specially qualified in epidemiology as assistants to the division sanitary inspector.
- (10) Provision for laying the dust in certain camps.
- (11) Special recommendations indicated by the local conditions at certain camps.

—V. C. V.

## *The Use of Atropine as a Diagnostic Agent in Typhoid Infections*

THE British Medical Research Committee has issued a monograph on this subject prepared by Captain Marris. The production of immunity to the typhoid infections by triple inoculation, now compulsory in all armies and largely practiced in civilian life, has not been altogether an unmixed blessing, brilliant as the results have been. The immunity attained is only relative and temporary, and, when infection occurs among the vaccinated, the diagnostic difficulties are greatly increased. The symptoms are generally mild and are often wanting in those features which characterize typhoid infections in the unvaccinated. It is true that a positive diagnosis may be made when the isolation and identification of the infecting bacillus is possible, but reliance upon agglutination tests is not safe.

"The atropine test due to Captain Marris, and now to be described, comes, therefore, at a most opportune time, when every additional aid to the diagnosis of this group of cases is to be welcomed. It will be seen that it has both advantages and disadvantages. Its advantages are its great simplicity, for the test can be performed at the bedside by an intelligent nurse, and the fact that it can be effectively applied earlier in the course of the fever than the agglutination test. A disadvantage is that it does not discriminate between the three types of infection, those due to *B. typhosus* and paratyphosus A and B respectively."

It is to be noted that these tests were applied to young soldiers and during the period between the fifth and fourteenth days of the disease. There seems no reason why the observations should not hold equally good in civilian practice. They have, however, a scientific, as well as a practical, interest. The results appear to be due to the antagonistic action between the alkaloid and the chemical poison developed in the course of typhoid infection.

"When the human body is so infested by bacilli of the typhoid group as to exhibit typhoid, paratyphoid A, or paratyphoid B fever, a toxin is formed which affects the heart in a peculiar manner; the presence of this toxin can be detected by observing the abnormal yet characteristic reaction of such hearts to certain drugs, notably atropine."

The principle of the test seems to depend upon the following known facts:

1. Under ordinary conditions the administration of atropine markedly increases the rate of heart beat.
2. Bradycardia is generally observed in typhoid infection.
3. In this infection the administration of atropine fails to increase the heart beats proportionately to the increase observed in health or in other diseases.

The application of the test is as follows:

"The patient lies horizontally and is instructed to remain completely at rest throughout this test, which is not employed until at least one hour has elapsed from the last meal. The pulse rate is counted, minute by minute, until it is found to be steady; ten minutes of such counting usually suffices. Atropine sulphate is then injected hypodermically in the dose of  $\frac{1}{32}$  grain, preferably over the triceps region to insure rapid absorption. An interval of 25 minutes is allowed to elapse,

and the pulse is again counted, minute by minute, until it is clear that any rise which may follow the injection has passed off; 15 or 20 minutes may be necessary for this purpose when the pulse rate is raised at the first count."

For instance, the average before the injection is 68 and that after the injection is 94, giving a difference of 26 beats. In this case the conclusion is reached that the infection is not typhoid. When the increase is not greater than 14 beats, typhoid infection is indicated. In the first instance the reaction is said to be negative, and in the second, positive. Experience fixes the upper limit of a positive reaction at 14 beats per minute and the lower limit of a negative reaction at 15 beats per minute.

"If the patient is admitted during the first fortnight of his illness, the test is applied as soon as possible after admission and is charted with the temperature. When a positive reaction (little or no response to atropine) is obtained, the diagnosis of infection with a member of the enteric group of organisms may be made. In the case of a negative reaction, the test should be repeated after two or three days, and, if again negative, it is again repeated. Three negative reactions falling within the first fortnight of the illness exclude the presence of typhoid with a considerable degree of certainty; there are rare exceptions and in these a continuation of the test is usually suggested by the symptoms and remaining clinical signs."

True cases of typhoid group which are admitted after the fourteenth day frequently yield positive reactions, but negative reactions after this period of illness are often unreliable.

The effect of  $\frac{1}{33}$  of a grain of atropine sulphate in the normal individual is as follows:

A slight and short fall in pulse rate is usually seen, and this is followed by a steep rise within ten minutes of the injection; the rise is constant, and is rarely less than 20 beats, and is frequently as much as 30 beats per minute in extent. It reaches its maximum at about 30 minutes, is maintained for varying periods, and then gradually subsides, reaching normal after an hour or more. Dryness of the mouth occurs soon after the injection. Dilatation of the pupil does not always appear but usually occurs after the lapse of an hour or more. A rise of blood pressure may be present, but is insignificant, being usually no greater than 5 or 6 mm. of mercury.

It may be desirable to report that this test has so far been used solely on soldiers who have previously been inoculated, and in whom typhoid is modified. As a rule the course of this modified typhoid is mild, the death rate not being more than 2 per cent. Of the symptoms the author writes:

"The patients are listless; there is mental hebetude; but delirium and the 'typhoid state' are rare. The face is flushed, the conjunctiva congested, and the expression one of fatigue. The tongue is moist and often furred, but its appearance can not be regarded as characteristic. The pulse is slow, soft, but of fair volume; it is not often dicrotic. The heart's apex beat is ill defined or absent; the sounds are clear, but distant. The abdomen is tumid or doughy and usually a little tender, especially in the left hypochondrium. The spleen may or may not be palpable. The eruption is infrequent, but it may be seen as isolated rose spots or rarely as a rash of protean character. The abdominal reflexes are generally

absent. Over the lungs moist sounds are frequent; signs of some consolidation, more often at the right base than the left, are not infrequent. The temperature may vary considerably, the course may be afebrile from admission, or from a day or two subsequent to admission; in some cases the curve falls abruptly to normal within a few days of admission and remains there, a type which is especially frequent when there have been signs of consolidation of the lungs. It may fall by lysis, ending relatively early at or about the tenth or fourteenth day of the disease. Spiky charts with evening rise and irregular charts resembling those of trench fever are not uncommon. Relapses and complications are comparatively rare."

In 111 cases in which the diagnosis was determined by blood cultures the atropine test was reliable in 98 per cent. The average pulse rise in these cases was 6.6 beats per minute. In 247 cases, diagnosed as typhoid infection by agglutination test, the atropine test was positive in 222.

The atropine test proved positive in a few individuals within from one to three days after typhoid inoculation.

The author attributes the atropine test to antagonistic action between this alkaloid and the typhoid poison on the inhibitory mechanism of the heart. There is no systemic antidotal value in atropine in typhoid fever and this drug is of no value in the treatment of the disease.

—V. C. V.



201

# *The Journal of Laboratory and Clinical Medicine*

VOL. III.

ST. LOUIS, FEBRUARY, 1918

No. 5

## ORIGINAL ARTICLES

### METHODS OF CONTROL OF THE CLOTHES LOUSE [PEDICULUS HUMANUS (VESTIMENTI)]\*

BY WILLIAM MOORE, ST. PAUL, MINN.

#### INTRODUCTION

LATE in April, 1917, at the suggestion of A. D. Hirschfelder and with the approval of the local branch of the National Research Council, I undertook a study of the methods of control of the clothes louse.† Peacock<sup>1</sup> has very clearly defined the problem, and from his description of conditions, there appear four possible methods which might be adopted for the destruction of the lice. These may be briefly summarized as; first, the destruction of the lice by the vapor of a volatile organic compound worn by the soldier as a sachet; second, the use of louse powders; third, impregnation of the underwear with an insecticide; fourth, the destruction by heat or fumigation of both lice and eggs in the garments, while the soldier is bathing.

#### DESTRUCTION BY MEANS OF SACHETS

Naphthalene,<sup>2</sup> camphor,<sup>3</sup> sulphur,<sup>4</sup> paradichlorbenzene,<sup>5</sup> and various other chemicals<sup>6</sup> have been recommended to be worn in small bags about the neck or the waist of the soldier. The principle of the sachet is the production of a poisonous vapor or gas in the space between the body of the soldier and his outer garments. Actual trials, of these and many other chemicals of about the same volatility, in our experiments show that lice will continue to live under such conditions and will even lay eggs. In some other investigations<sup>7, 8</sup> I have shown that the toxicity of volatile organic compounds to insects increases as the boiling

\* Published, with the approval of the Director, as Paper No. 105 of the Journal Series of the Minnesota Experiment Station.

† I wish to express my thanks to S. A. Graham and Miss Anna Wentz for assistance in the care and rearing of the lice used in the experiments.

point rises and the volatility decreases. Compounds with a boiling point higher than  $225^{\circ}$  C. in general are so slightly volatile that not sufficient vapor is produced to kill the insects within a reasonable period of time. Theoretically, therefore, such compounds as naphthalene, camphor, and paradichlorbenzene should be the best insecticides for use in the destruction of lice. The failure of these compounds must, therefore, be due either to the lack of diffusion or the rapidity with which the vapor escapes. In large glass containers where there is no opportunity of escape, the vapor diffuses in a few hours sufficiently to kill lice in all parts of the flask. It appears, therefore, that the ineffectiveness of these compounds must be due to the rapidity with which their vapors escape through the clothing. The following experiments were conducted to determine the accuracy of this point.

Five-gram lots of the compounds to be tested were weighed out in watch glasses and placed in wide-mouthed jars lying on their sides. The openings of the jars were covered with different types of cotton, wool, and silk underwear, woolen and khaki clothes, and combinations of these to as many as three or four thicknesses. A control jar was left open while another control was closed with a glass top. From a comparison of the amount of evaporation from these jars, it is possible to arrive at an estimation of the amount of diffusion through the different types of clothing. In the case of the jar closed with a glass top, the air soon became saturated with the vapor after which no further evaporation occurred. This amount was so small in the case of camphor, naphthalene, and paradichlorbenzene, that no difference in weight could be detected in the com-

TABLE I

EVAPORATION OF VOLATILE COMPOUNDS FROM JARS CLOSED WITH DIFFERENT KINDS OF CLOTH

NAPHTHALENE	
Check,	100%
Light cotton gauze underwear,	66%
Medium cotton and wool underwear,	88%
Medium cotton underwear,	66%
Medium silk and cotton underwear,	66%
Medium silk underwear,	88%
Heavy cotton fleece-lined underwear,	88%
Heavy cotton and wool underwear,	85%
Blue flannel,	77%
Double blue flannel,	44%
Muslin underwear, woolen shirt, khaki coat,	50%
PARADICHLORBENZENE	
Check,	100%
Medium wool and cotton underwear,	62%
Fleece-lined cotton underwear,	55%
Light cotton gauze underwear,	60%
XYLENE	
Check,	100%
Fleece-lined cotton underwear,	46%
Light gauze cotton underwear,	50%
Medium silk underwear,	50%
Medium wool and cotton underwear,	42%
Heavy cotton underwear,	46%
Muslin underwear, woolen shirt, khaki coat	43%

pound. Evaporation from the open jar was considered as 100 per cent and on this basis it was found that naphthalene diffusing through different types of clothing varied from 44 to 88 per cent, and paradichlorobenzene, from 55 to 62 per cent (see Table I).

From these results it is apparent that not more than 15 to 50 per cent of a saturated atmosphere of these compounds could be retained under the soldier's uniform; or, in other words, the vapor escapes the clothing almost as rapidly as the compound evaporates. Such a low percentage of saturation is not sufficiently high to result in effective control of the lice. Similar experiments conducted with a more volatile compound such as xylene mixed with fuller's earth showed a leakage of from 40 to 45 per cent. Fifty per cent of a saturated atmosphere of xylene could thus be maintained, and this quantity is sufficient to produce the death of lice. In actual experience, where the lice were not confined in a vial, it was found that they were merely stupified and lodged in the lower portions of the trouser legs, where, not sufficient vapor being present, they revived. To overcome this difficulty sachets might be attached about the knees on both legs, but when the large quantity of xylene which is necessary to maintain the desired degree of saturation is considered, it is at once apparent that such treatment is eliminated by its expense.

As a summary of the results of the work with sachets, it might be stated that compounds such as naphthalene, camphor, or paradichlorobenzene do not volatilize rapidly enough to make good the loss incurred by leakage through the clothing. Compounds with lower boiling points such as xylene mixed with fuller's earth might be used successfully as sachets, but the expense of treatment is prohibitive.

#### LOUSE POWDERS

The most successful louse powders in use at the present time contain naphthalene, and the most favored on the Western front seem to be NCI<sup>1</sup> powder made of naphthalene, 96 per cent; creosote, 2 per cent; iodoform, 2 per cent. The chief objections to this powder are:<sup>9</sup> first, that it is moist, and hence difficult to dust through the clothing, and, second, it is inclined to burn the skin particularly in the fork of the legs.

In the studies of Kinlock<sup>9</sup> and in my own work it was found that the combined powder was more toxic to the lice than any one of its constituents. Experiments show that a saturated solution of naphthalene in creosote was more toxic than creosote alone. The same is true of a saturated solution of iodoform in creosote. Creosote saturated with both iodoform and naphthalene is more toxic than creosote alone, but not as toxic as either creosote-naphthalene, or creosote-iodoform. Just as in fractional distillation, the lower fraction of the liquid, carries over with it small quantities of the higher boiling liquid, so in evaporating, the creosote carries over portions of the naphthalene or iodoform. This fact appears to be the underlying principle which governs the toxicity of the NCI powder.

One cubic centimeter of creosote in evaporating will never carry more than one-half gram of naphthalene or one-fourth gram of iodoform, hence there is at least 95 per cent more naphthalene in the NCI mixture than is required. In

the experiments where talc was substituted for a large part of the naphthalene, a dry powder, easy to dust through the clothing was obtained, which powder retained its dryness even when a much larger percentage of creosote was added. Using talc as a basis, a number of new powders were made up containing creosote or a liquid of about the same volatility and a slightly volatile solid.

TABLE II

COMPOSITION OF POWDERS	PER CENT KILLED IN			
	5 MIN.	10 MIN.	20 MIN.	30 MIN.
NCl (naphthalene 96%, creosote 2%, iodoform 2%)			66	100
Talc 20 grams, naphthalene $\frac{1}{2}$ gram, creosote 1 c.c., iodoform $\frac{1}{2}$ gram			66	100
Talc 20 grams, creosote, 1 c.c.			0	100
Talc 20 grams, creosote 1 c.c., naphthalene $\frac{1}{2}$ gram		66	100	100
Talc 20 grams, creosote 1 c.c., iodoform $\frac{1}{2}$ gram		0	100	100
Talc 20 grams, creosote $\frac{1}{2}$ c.c., oil of sassafras $\frac{1}{2}$ c.c.				0
Talc 20 grams, creosote $\frac{1}{2}$ c.c., amyl alcohol, $\frac{1}{2}$ c.c.			0	100
Talc 20 grams, creosote $\frac{1}{2}$ c.c., methyl salicylate $\frac{1}{2}$ c.c.			0	100
Talc 20 grams, creosote $\frac{1}{2}$ c.c., carbolineum $\frac{1}{2}$ c.c.			0	0
Talc 20 grams, creosote $\frac{1}{2}$ c.c., crude phenol $\frac{1}{2}$ c.c.	66	100	100	100
Talc 20 grams, creosote 1 c.c., chloretone $\frac{1}{2}$ gram		33	100	
Talc 20 grams, methyl salicylate 1 c.c., chloretone $\frac{1}{2}$ gram			0	
Talc 20 grams, creosote 1 c.c., alpha-naphthylamine $\frac{1}{2}$ gram			66	
Talc 20 grams, methyl salicylate 1 c.c., alpha-naphthylamine $\frac{1}{2}$ gram			33	
Talc 20 grams, creosote 1 c.c., paranitrophenol $\frac{1}{2}$ gram		33	100	
Talc 20 grams, methyl salicylate 1 c.c., paranitrophenol $\frac{1}{2}$ gram			66	
Talc 20 grams, methyl salicylate 1 c.c., iodoform $\frac{1}{2}$ gram			0	
Talc 20 grams, methyl salicylate 1 c.c., naphthalene $\frac{1}{2}$ gram			0	
Talc 20 grams, methyl salicylate 1 c.c., quinone $\frac{1}{2}$ gram		100	100	
Talc 20 grams, methyl salicylate 1 c.c., paranitrobenzylchloride $\frac{1}{2}$ gram			33	
Talc 20 grams, creosote 1 c.c., ortho- and parachloronitrobenzene, $\frac{1}{2}$ gram		66	100	
Talc 20 grams, creosote 1 c.c., picric acid $\frac{1}{2}$ gram			0	
Talc 20 grams, creosote 1 c.c., alpha-naphthol $\frac{1}{2}$ gram		66	100	
Talc 20 grams, creosote 1 c.c., beta-naphthol, $\frac{1}{2}$ gram		66	100	
Talc 20 grams, creosote 1 c.c., paradibrombenzene $\frac{1}{2}$ gram	66	100	100	
Talc 20 grams, creosote 1 c.c., menthol $\frac{1}{2}$ gram			66	
Talc 20 grams, creosote 1 c.c., monochloroacetic acid $\frac{1}{2}$ gram	100	100	100	
Talc 20 grams, creosote 1 c.c., chloranil $\frac{1}{2}$ gram			0	
Talc 20 grams, creosote 1 c.c., sulphur $\frac{1}{2}$ gram	100	100	100	
Talc 20 grams, creosote 1 c.c., cumarin $\frac{1}{2}$ gram		66	100	
Talc 20 grams, creosote 1 c.c., camphor $\frac{1}{2}$ gram	0	100	100	
Talc 20 grams, creosote 1 c.c., isoborneol $\frac{1}{2}$ gram	66	100	100	
Talc 20 grams, creosote 1 c.c., monobromated camphor $\frac{1}{2}$ gram	66	100	100	
Talc 20 grams, crude phenol 1 c.c., naphthalene $\frac{1}{2}$ gram		0		
Talc 20 grams, crude phenol $\frac{1}{2}$ c.c., creosote $\frac{1}{2}$ c.c., naphthalene $\frac{1}{2}$ gram		66		
Talc 20 grams, creosote 1 c.c., naphthalene and sulphur to saturation	33	100		
Talc 20 grams, creosote 1 c.c., sulphur to saturation	66	100		

A small piece of cloth bearing the lice was placed on a small glass plate and then covered over with a piece of underwear dusted with the powder to be tested. Comparative tests between lice treated on the arm and on the glass plate showed no difference in the period of time required to kill them. Results of these experiments are tabulated in Table II.



Liquids less volatile or more volatile than creosote are not as successful. Methyl salicylate and crude phenol are probably the best substitutes, but not quite as good. No advantage was noted in the use of a compound which readily sublimates such as iodoform, chloranil, and quinone. Considered from the standpoint of irritation to the skin, ease of dusting through the clothing, effectiveness, and expense, the powder recommended consists of creosote 1 c.c., sulphur  $\frac{1}{2}$  gram, and talc 20 grams. This powder could be made up for from 5 to 10 cents a pound and will kill 100 per cent of the lice in 5 minutes. A few lice may show signs of life at the end of five minutes, but will later die even though removed from the powder.

Two points may be mentioned concerning the use of powders to destroy the lice. The soldier in general objects to insect powders, since effective powders are inclined to produce irritation, particularly when the soldier is perspired, while powders which do not burn are of no value. The second point is the enormous quantity of powder which would be necessary to effectively treat the great numbers of men at the front. Using two ounces of powder every day or two, which quantity would be necessary for a thorough job, would mean 1250 pounds to each division of ten thousand men. The use of a powder can only be considered as a supplementary control measure.

#### IMPREGNATION OF UNDERWEAR

Impregnation of the underwear with active volatile organic compounds would, if the amount of the chemical were large, result in considerable irritation of the skin, greater in fact, than that accompanying the use of louse powders, while smaller quantities would soon evaporate and be ineffective. It might be noted that a number of essential oils have been used with greater or less success.<sup>10, 11, 12, 13</sup> The more volatile essential oils are liable to cause considerable burning of the skin while the less volatile essential oils have but slight advantage over the fixed vegetable oils. Slightly volatile mineral, vegetable and animal oils were studied. The lice are inclined to leave cloth impregnated with these oils, but if forced to remain on them they will die.

Olive oil killed 50% in 4 days.
Cedar oil killed 100% in 20 hours.
Castor oil killed 100% in 92 hours.
Cod-liver oil killed 100% in 68 hours.
Paraffin oil killed 100% in 68 hours.
Liquid petrolatum killed 100% in 20 hours.
Rape-seed oil killed 100% in 44 hours.
Neat's-foot oil killed 50% in six days.
Lard oil killed 100% in 116 hours.
Cotton seed oil killed 50% in 4 days.
Commercial oleic acid killed 100% in 20 hours.
Raw linseed oil killed 100% in 63 hours.
Boiled linseed oil killed 100% in 63 hours.
Whale oil killed 100% in 63 hours.
Fish oil killed 100% in 15 hours.
Peanut oil killed 100% in 39 hours.

From an insecticide standpoint the results are not very favorable although all the oils were used without dilution. Oils might be used as repellants but

underwear thus treated is oily or greasy and loses its absorption properties. The value, therefore, of impregnating the underwear with slightly volatile oils is questionable.

An arrangement by which the soldier would wear outside of his underwear a cheesecloth pajama suit impregnated with a volatile organic compound might be feasible. For this purpose a liquid<sup>14</sup> has the advantage over a solid which would require a solvent in its application and would make the cloth stiff and harsh. Cheesecloth dipped in a saturated solution of sulphur in creosote and wrung out nearly dry is successful. The vapor from the impregnated cheesecloth penetrates through the underwear and kills the lice in from twenty to thirty minutes. Suits could be made here and dipped near the front. Comparatively little of the solution would be used if the suits, after dipping, were passed through a strong wringer. Such suits could be redipped and would be as valuable as a louse powder without as much waste of material and without the danger of skin irritation.

#### FUMIGATION

Heat has been used to a large extent in destroying lice and eggs in the clothing, while the soldier is taking a bath.<sup>15, 16, 17</sup> Dry heat must be carefully watched and regulated to prevent scorching of the clothing.

140° C. of dry heat kills lice and eggs in about 30 minutes.

160° C. of dry heat kills lice and eggs in about 10 to 15 minutes.

In practice it is difficult to reach so high a temperature and maintain it with a large quantity of clothing. The time available for the work is usually stated as about 15 to 20 minutes. Steam leaves the garments wet at the close of the treatment and is apt to ruin leather. Carbon bisulphide, ammonia, sulphur dioxide<sup>18, 19, 20</sup> and hydrocyanic acid are not able to penetrate all parts of the clothing in the short period allowed, while formaldehyde is but slightly toxic to insects. As previously stated, the volatile organic compounds with the lower boiling points are the least toxic.<sup>7, 8</sup> One chemical, however, is a notable exception. Chlorpicrin or nitrochloroform ( $\text{CCl}_3\cdot\text{NO}_2$ ) although quite volatile, is very toxic. It also has great penetration. Experiments conducted with this chemical have shown that clothing can be piled into a galvanized iron ash tin, sprinkling chlorpicrin through them at the rate of 4 c.c. to the cubic foot and in fifteen minutes all lice will be dead, while a half hour's fumigation is sufficient to kill eggs in all stages of development. In order to kill the eggs by fumigation in thirty minutes, a small amount of heat is necessary to hasten the evaporation of the chlorpicrin. To each cubic foot of space one liter flask filled with water heated to 80° C. was used in the experiments. A twenty-minute fumigation will destroy a large number of the eggs, particularly the youngest and oldest.

In actual practice the outer garments of the men can be fumigated while they are bathing. Ash tins, or perhaps better, a collapsible metal box such as is used on the Italian front for fumigation<sup>21, 22</sup> could be used as a container. Heated stones placed in the fumigation box or even a heated fumigation chamber might be used. By increasing the heat, the period necessary to kill the eggs could be reduced to fifteen minutes. One to two uniforms may be packed in

each cubic foot and the vapor will still be able to penetrate. Arrangements for opening the box outside would be necessary due to the irritation of the chemical to the eyes, nose, and throat. The operator could wear a gas mask when opening the fumigation box and airing the clothing. Airing for three minutes in the open is sufficient to remove the vapor from the clothing.

By a well organized system of allowing the soldier a bath every week or two, at which time they would receive clean underwear and shirts, while their outer garments were fumigated, together with the use of impregnated suits, or a good louse powder as a supplementary measure to prevent reinfestation, it should be possible to rid the men of lice. The destruction of the lice and eggs in the dirty garments could be accomplished by washing and soaking in a water containing a good insecticide.<sup>23, 24, 25</sup>

In conclusion it might be noted that the object of this paper is to give certain facts which have been ascertained concerning the toxicity of various chemicals to lice. Although several methods of destroying lice have been pointed out, they should not be considered as the solution of the louse problem. The real solution of the problem will rest in the practical application of such principles as are determined in the laboratory, and the success of the application will depend upon the thoroughness of the organization developed for carrying out the work.

#### SUMMARY

Sachets are not successful.

Talc 20 grams, creosote 1 c.c., sulphur  $\frac{1}{2}$  gram is six times as effective a louse powder as NCI, causing less irritation to the skin and, being dry, is easier to apply.

Impregnation of the underwear does not appear promising, but a cheese-cloth suit impregnated with saturated solution of sulphur in creosote could be successfully worn outside the underwear.

Chlorpicrin can be used as a fumigant, penetrating the clothing and killing the lice in all parts of the clothing in fifteen minutes and the eggs in thirty minutes. By increasing the heat in the fumigation chamber, the time required to kill the eggs could be reduced.

#### BIBLIOGRAPHY

- <sup>1</sup>Peacock, A. D.: The Louse Problem at the Western Front, *Brit. Med. Jour.*, May 27 and June 3, 1916, 745-749, 784-788.
- <sup>2</sup>Noel, P.: Les mouches, les moustiques, les poux et les rats dans les tranchées, *Bull. Trim. Lab. Entom. Agric. Seine Infér.* Rouen, Jan., Feb., and March, 1916, 9-15.
- <sup>3</sup>Instructions for the Destruction of Clothes Lice, *Bull. All Russian Union of Towns*, Moscow, October, 1915, No. 18, 58-60.
- <sup>4</sup>Shipley, A. E.: Insects and War, *Brit. Med. Jour.*, Sept. 19, 1914, and Nov. 14, 1914.
- <sup>5</sup>Hase, A.: Weitere Beobachtungen über die Läusephage, *Centralbl. f. Bakteriol.*, Nov. 29, 1915, lxxvii, No. 2, 153-163.
- <sup>6</sup>Postnikov, A. I.: On the Question of the Control of Lice in the Active Army, *Proceedings of the Conference of Bacteriologists and Representatives of Medical Sanitary Authorities on the Campaign against Infectious Diseases in Connection with the War*, Moscow, Jan., 1915, 70, 71.
- <sup>7</sup>Moore, W.: Toxicity of Various Benzene Derivatives to Insects, *Jour. Agr. Research*, 1917, ix, No. 11, 371-381.

- <sup>8</sup>Moore, W.: Volatility of Organic Compounds as an Index of the Toxicity of Their Vapors to Insects, *Jour. Agr. Research*, 1917, x, No. 7, 365-371.
- <sup>9</sup>Kinloch, J. P.: An Investigation of the Best Methods of Destroying Lice and Other Body Vermin, *Brit. Med. Jour.*, June, 1916, 789-793.
- <sup>10</sup>Lobaczewski, A. R.: Zur Frage der Entlausung, *Wien. klin. Wchnschr.*, April, 1915, xxviii, No. 14, 373, 374.
- <sup>11</sup>Sergeant, E., and Foley, H.: Destruction par l'essence d'eucalyptus des poux du corps, agents transmetteur de la fièvre récurrente et du typhus exanthématique, *Bull. Soc. Path. Exot.*, Paris, June, 1915, viii, No. 6, 378-381.
- <sup>12</sup>Legroux R.: Sur la destruction des poux, *Bull. Soc. Path. Exot.*, Paris, 1915, viii, No. 7, 470-473.
- <sup>13</sup>Galewsky: Zur Behandlung und Prophylaxe der Kleiderläuse, *Deutsch. med. Wchnschr.*, 1915, 285.
- <sup>14</sup>Gunn, J. A.: A Note on the Prevention of Pediculosis, *Brit. Med. Jour.*, May, 1917, No. 2940, 579, 580.
- <sup>15</sup>Bacot, A. W.: The Temperature Necessary for the Destruction of Lice and their Eggs, *Brit. Med. Jour.*, Jan., 1916.
- <sup>16</sup>Heymann, B.: Die Bekämpfung der Kleiderläuse, *Bull. Inst. Pasteur*, Paris, March, 1916, xiv, No. 6, 191.
- <sup>17</sup>Legendre, J.: Destruction des poux de corps par le cresyl et le brossage, *Bull. Soc. Path. Exot.*, Paris, May, 1915, viii, No. 5, 280-283.
- <sup>18</sup>Friedmann, A.: Beiträge zur Bekämpfung der Kleiderläuse in Kleidern, *Centralbl. f. Bakteriol. alt. originale*, lxxvii, Part 4, 320-338.
- <sup>19</sup>Widmann, E.: Beiträge zur Kenntnis der Biologie der Kleiderläuse und deren Bekämpfung, *Bull. Inst. Pasteur*, Paris, March, 1916, xiv, No. 6, 190.
- <sup>20</sup>Galewsky: Vorschläge zur Entlausung von Gefangenenlagen, *Deutsch. med. Wchnschr.*, May, 1915, xli, No. 22, 652, 653.
- <sup>21</sup>Alessandrini, G.: I pidocchi ed i mezzi per distruggerli. *Annali d' Igiene*, Rome, Feb., 1916, xxvi, No. 2, 92-108.
- <sup>22</sup>Muto, A.: Nuovo Metod di Sterilizzazione Entomo.-parassitario, *Ann. d' Igiene*, Rome, August, 1916, xxvi, No. 8, 493-508.
- <sup>23</sup>Kinloch, J. P.: An Investigation of the Best Methods of Destroying Lice and Other Body Vermin, *Brit. Med. Jour.*, June, 1915, No. 2842, 1040, 1041.
- <sup>24</sup>Bacot, A. W.: The Louse Problem, *Proc. Roy. Soc. Med.*, London, 1917, x (Sec. of Epidemiology and State Medicine), 61-94.
- <sup>25</sup>Kisskalt, K.: Die Bekämpfung der Läuseflage, *Deutsch. med. Wchnschr.*, 1915, 154.



## THE ETIOLOGY OF SCARLET FEVER\*

### I. A STUDY OF ORGANISMS FOUND IN THE BLOOD OF SCARLET FEVER PATIENTS

BY R. W. PRYER, D.P.H., AND J. B. KELLY, M.S., DETROIT, MICH.

THE city of Detroit, as well as many other cities of the central portion of the United States, had an unusually high number of scarlet fever cases during the latter part of the winter and early spring of this year.

At one time there were 1385 cases under quarantine in Detroit. The population of Detroit, according to the United States census method of estimation, is 571,784. However, there is no doubt that the true population of the city is in the neighborhood of 750,000. Even this figure shows a tremendously high case rate per thousand of population.

No one organism has been accepted as the cause of scarlet fever, although a vast amount of work has been done on this disease and many suppositions advanced as to the infective agent.

Chief among these is the theory that the causative organism is a streptococcus. This theory is based largely on the fact that members of this family are usually found present in the throat of scarlet fever patients. However, it has not been definitely proved that streptococci are the cause of the disease.

Other workers have claimed that a protozoon is the infective agent. Still others have thought that the organism causing the disease is a virus of the filterable type, but again definite proof is lacking.

An English worker<sup>7</sup> has carried out an extended piece of work which leads him to conclude that a diplococcus is the infecting organism in scarlet fever; but we are of the opinion that he has not entirely differentiated between what he calls the diplococcus scarlatinae and the pneumococcus.

More recently Mallony and Medlar<sup>8</sup> have reported the results of an investigation on scarlet fever and describe a bacillus as being the infective agent. They give to this organism the name bacillus scarlatinae.

The first part of this paper deals with only one of several organisms that we have isolated from the blood of scarlet fever patients. We are doubtful about this organism having anything to do with the disease, but are reporting it in detail in order to show the presence of organisms of the diphtheroid group in the blood of scarlet fever patients, of normal individuals, and of persons sick with various diseases.

Since there was an abundance of clinical material for our purpose, we have worked only with the blood of cases which showed typical symptoms of this disease, and the cases reported here were bled during the period in which there was a marked rash.

These cases were diagnosed scarlet fever by the attending physician and

\*From the Laboratory of the Detroit Board of Health.

the diagnosis concurred in by Dr. Ostrander, resident physician at the Detroit Municipal Hospital.

#### METHOD OF BLEEDING

Patients were bled from one of the veins at the bend of the elbow by means of a sterile Luer syringe, 6 to 8 c.c. of blood being withdrawn. The skin over the area of puncture was treated in one of three ways:

1. Scrubbed with strong soap, then washed with 95 per cent alcohol, and finally painted with tincture of iodine.
2. Soaked for five minutes in a 1:1000 mercuric chloride solution.
3. Painted with tincture of iodine alone.

The third method seems to be as satisfactory as any, and is the one recommended by most workers in this field.

#### METHOD OF MAKING CULTURES

Three methods were used in making the cultures from the blood.

1. One cubic centimeter of blood was added to tubes of sterile broth made up as follows:

2 tubes of nutrient bouillon, plus 10 per cent tryptic extract.

2 tubes of nutrient bouillon, plus 10 per cent tryptic extract,  
plus 4 per cent glycerin.

2 tubes of nutrient bouillon, plus 10 per cent tryptic extract,  
plus 1 per cent dextrose.

One of each was covered with  $\frac{1}{2}$  inch of paraffin oil, the other maintained under aerobic conditions.

2. The blood was mixed with an equal quantity of tryptic extract and plates made as follows:

One-half c.c., 2 c.c., and 5 c.c. of mixture plated in duplicate, using agar which was neutral to phenolphthalein. Half of plates were incubated aerobically; the other half, anaerobically in an atmosphere of hydrogen.

3. The blood was divided into two portions: One portion added to tryptic extract as before, the other portion being mixed with the minimum amount of sodium oxalate solution which prevents clotting. One-half c.c. and 2 c.c. plates were made in duplicate from each mixture.

These cultures were all incubated at 37° C. for a period of two to three weeks. Tubes showing signs of growth were transplanted on to blood agar slants and on to Loeffler's media. Plates were examined every forty-eight hours and were not opened unless we desired to examine a particular colony, in which case transplants were made as above.

#### NUMBER OF CASES IN WHICH DIPHTHEROID WAS FOUND

The first nine cases studied were all scarlet fever, and we found that it was possible to isolate a diphtheroid organism from the blood in a majority of the cases. It was soon found, however, that the same thing held true for other diseases and for normal individuals. In all, twenty-four cases of scarlet fever have been studied. These were all in the acute stage of the disease. In nineteen, or 79 per cent, of these cases this diphtheroid organism has been isolated in pure culture.

In six normal individuals a diphtheroid organism, morphologically and culturally identical with the one isolated from the nineteen cases of scarlet fever, has been isolated from the blood of four.

In cases of disease other than scarlet fever this organism was found as follows:

Measles, one case, diphtheroid isolated.

Diphtheria, five cases, diphtheroid isolated in two cases.

Pneumonia, four cases, not found.

Typhoid fever, one case, not found.

#### MORPHOLOGY OF ORGANISM

This diphtheroid is a very pleomorphic organism, varying from coccus forms to a fairly long bacillus. Our experience with this organism has been similar to

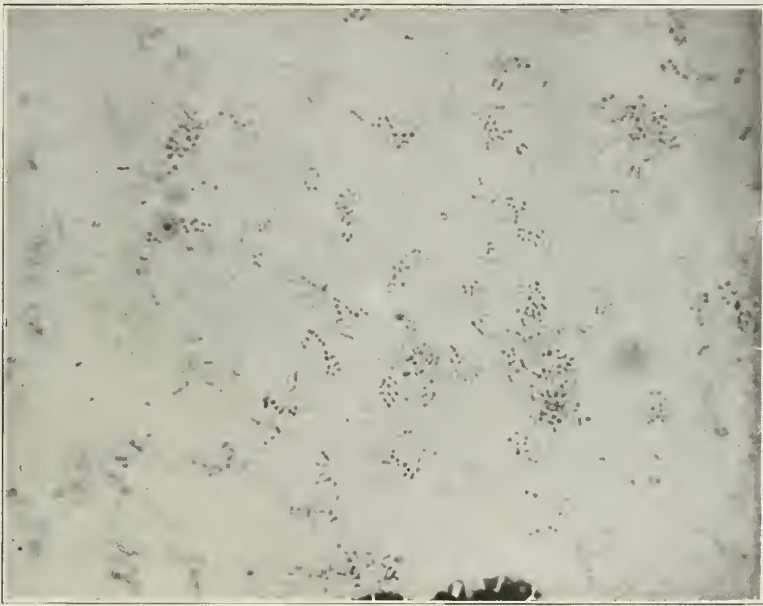


Fig. 1.

other workers with diphtheroids of this class, in that our first cultures were plated many times in order to separate the cocci from the bacilli, and all the time we were working with a pure culture.

In older cultures coccus forms predominate, while in young (18- to 24-hour) cultures the bacillus forms predominate.

The bacilli are usually of the barred type and tend to be shorter and plumper than *B. diphtheriæ*. This is particularly true if they are grown in media containing serum. However, bipolar staining forms, very similar to *B. diphtheria*, are frequently seen; and these forms are observed more frequently in young cultures on nutrient agar.

The organism stains readily with the ordinary stains. It is strongly gram-positive and is slightly acid-fast. Loeffler's methylene blue is the best stain. No spores can be demonstrated by any of the staining methods; no motility was observed.

Fig. 1 shows a preparation made from a culture eight days old. It will be noted that the coccus forms predominate.

This organism is nonpathogenic for mice, rats, guinea pigs, and rabbits. Its pathogenicity for monkeys has not been tested.

No agglutination has been observed either with serum of scarlet fever patients or normal serum.

Ten minutes at 70° C. kills this organism, provided an even emulsion is used in making the test.

#### CULTURAL CHARACTERISTICS

This organism grows readily on solid media at 37° C., after the first culture is obtained. Frequently the plates made with the blood direct require nearly three weeks before the colonies of the diphtheroid can be found. After the first transplant is made, the growth at eighteen hours is heavy.

Growth at room temperature is very slow and scanty.

Growth on gelatin is very slow, media not liquefied.

The growth in liquid media is slow, but heavy after several days' incubation.

No gas is formed in any sugar media. Acidity increases slightly in the following sugar media: dextrose, levulose, sucrose, lactose, maltose, dextrine and mannite.

The organism is a facultative anaerobe.

#### CONCLUSION ON DIPHTHEROID

There is no question that the diphtheroid group of organisms contains many different varieties. Mellon,<sup>1</sup> in a recent article, makes the statement that he believes there are forty-five different varieties of diphtheroids.

Fox<sup>2</sup> has pictured many different forms of these organisms.

Bunting and Yates<sup>3</sup> have reported an organism of this group as being the etiologic factor in Hodgkin's disease and call it *corynebacterium hodgkini*. The description of the organism reported by these men corresponds very closely with the organism reported in this paper.

#### A LARGE POLYMORPHIC ORGANISM ISOLATED FROM THE BLOOD OF A MAN DYING OF SCARLET FEVER

Blood cultures were made of one case of scarlet fever shortly before death. After about ten days' incubation, one colony of rather unusual appearance was found on one plate. Direct smears made from this colony, stained with Loeffler's methylene blue, showed what at first was considered a very large diplococcus. Cultures made from this developed a heavy growth after 24 hours in the incubator, blood agar being used for subcultures in this case. Smears made from these subcultures showed many bacillus-like forms as well as very large diplococci. The growth in these cultures being mucus-like, it was thought at first that we might be dealing with a member of the mucus capsulatus family. This was not the case, as a more thorough study of the cultural and morphologic characteristics of the organism plainly showed.



## MORPHOLOGIC AND STAINING CHARACTERISTICS

This organism varies from coccus-like forms to fairly long bacillary forms. Length, 2 to 8  $\mu$ . Breadth, 2 to 4  $\mu$ . It is nonmotile, and apparently does not form spores.



Fig. 2.

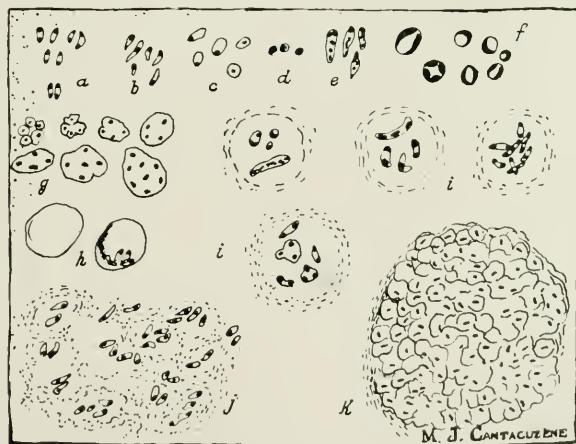


Fig. 3.

It stains readily with the simple stains and at first glance looks like a mixture of very large diplococci and bacilli staining heavily at each end and with a central faintly staining area.

With Giemsa's or Romanovsky's stain, the organism shows a beautiful differentiation, the ends staining bluish purple, a central unstained vacuole, and,

somewhere within this unstained area, a well-defined red spot which looks very much like a nucleus. This is shown in Fig. 2.

Older cultures show longer forms, the red spot lengthening and becoming more irregular in form.

#### CULTURAL CHARACTERISTICS

This organism grows best at 37° C., growth at room temperature being very much slower. Twenty-four hours at 37° C. on blood agar gives a heavy raised growth, mucus-like in appearance and regularly defined gray colonies. After several days in the incubator, the growth flattens out and is almost invisible, while the culture gives off a pronounced putrid odor.

Gelatin growth is poor; no liquefaction observed after three weeks.

In plain bouillon, growth is practically nil.

In sugar media, dextrose, levulose, maltose, mannite, sucrose, lactose, and dextrine, no gas is formed and after ten days the cultures are much less acid than at first.

#### PATHOGENICITY FOR ANIMALS

Rats, guinea pigs and rabbits are apparently unaffected by injection of this organism when made either intraperitoneally or subcutaneously. Mice are killed after about four days. Further work on this, as well as on toxin formation, is under way and will soon be reported.

#### SUMMARY

We realize that we have not proved that this organism is the cause of scarlet fever, but we do think that it is worthy of much further work. Class,<sup>4</sup> several years ago, described a diplococcus that he found in the throats of scarlet fever patients. Mallory<sup>5</sup> has described a protozoon which he found in the skin of scarlet fever patients. Cantacuzene<sup>6</sup> gives a very brief description of an organism found in the throat of a scarlet fever patient and claims to have produced in monkeys a disease very similar to scarlet fever by injection of these cultures.

From the description of Cantacuzene, the organism found by him in the throat is similar to, if not identical with, the one we have described, and which we isolated from the blood in a fatal case of scarlet fever. Fig. 3 is a reproduction of a cut in Cantacuzene's article, and many of these forms can be seen in the microphotographs shown by us in Fig. 2. This work will be continued.

#### BIBLIOGRAPHY

- <sup>1</sup>Mellon, R. R.: A Study of the Diphtheroid Group of Organism with Special Reference to their Relationship to the Streptococci. Part I. Characteristics of a Peculiar Pleomorphic Diphtheroid, *Jour. Bacteriol.*, ii, No. 2.
- <sup>2</sup>Fox, Herbert: Studies on Diphtheroid No. 111, *Arch. Int. Med.*, Sept. 15, 1915.
- <sup>3</sup>Bunting and Yates: An Etiologic Study of Hodgkin's Disease, *Jour. Am. Med. Assn.*, lxii, 516; *Ibid.*, lxi, 1803; *Ibid.*, August, 1913, 236.
- <sup>4</sup>Class, W. J.: The Diplococcus Scarlatinæ, *Illinois Med. Jour.*, Springfield, v, 77.
- <sup>5</sup>Mallory, F. B.: Scarlet Fever—Protozoon-like Bodies Found in Four Cases, *Jour. Med. Research*, x, 483.
- <sup>6</sup>Cantacuzene, M. J.: Sur un Microorganismes isole 'dans la Scarletine, *Compt. rend. Acad. d. sc.*, clix, 381.
- <sup>7</sup>Nair, W.: On the Etiology of Scarlet Fever, *Jour. Path. and Bacteriol.*, April, 1916.
- <sup>8</sup>Mallory and Medlar: The Etiology of Scarlet Fever, *Jour. Med. Research*, Nov., 1916.

# THE FACTORS OF COAGULATION IN THE BLOOD IN CERTAIN PATHOLOGIC CONDITIONS\*

BY DOROTHY FOSTER PETTIBONE, B.A., M.S.

*Mayo Foundation, Rochester, Minn.*

ELABORATE studies have been made in the past on the various factors which concern the coagulation of blood. Howell<sup>7</sup> has outlined minutely the individual constituents and their respective functions in normal blood. According to Howell, there are five factors in coagulation, that is, prothrombin, fibrinogen, calcium salts, thromboplastin and antithrombin, all of which may show variations in disease. These are all normally present in the circulating blood except thromboplastin, which is contained in all tissue juices, as well as in the formed elements of the blood. On injury of the vessel, therefore, thromboplastin is liberated from the surrounding tissue and the platelets and frees the blood of antithrombin, allowing the prothrombin to act with calcium and the thrombin to unite with fibrinogen and form fibrin. According to Morawitz (quoted by Drinker and Hurwitz),<sup>3</sup> thrombokinase transforms thrombogen, which normally circulates in the blood, to prothrombin. Prothrombin is then changed to thrombin by calcium salts. Thrombin reacts with fibrinogen and produces fibrin. Thrombogen has not been differentiated in any way from prothrombin.

Since the common acceptance of these underlying principles, much work has been done on these same factors in various pathologic conditions with special reference to hemorrhagic disturbances. Methods have been devised whereby these separate factors may be isolated and quantitatively estimated. I have studied a group of cases with special emphasis on prothrombin calcium salts, and platelets. Among these 45 cases, are 2 of hemophilia, 7 of myelogenous leucemias, 2 of purpura, 9 of epilepsy, 12 of jaundice (10 obstructive and 2 hemolytic) and 13 of a miscellaneous group.

## HEMOPHILIA

Many workers have reported observations on the blood of hemophiliacs, and the facts they note have agreed for the most part. Howell,<sup>8</sup> in his prothrombin tests, proved that one may by this comparative method readily distinguish between hemophiliac and normal bloods. Sahli believes that the condition is due to deficient thromboplastic material, but there has been suggested as yet no satisfactory method for determining the amount of this substance. The method I have used for determining prothrombin time, is that of Howell.<sup>8</sup> Draw 8 c.c. of blood into a sterile syringe, previously rinsed out with normal salt solution, and express into a tube containing 1 c.c. of a 1 per cent potassium oxalate. Invert the tube once to mix thoroughly and centrifugalize for fifteen minutes. In a series of small tubes place graduated amounts of 0.5 per cent calcium

\*Presented on May 28, 1917, in partial fulfillment of the requirement for the Degree of Master of Science, University of Minnesota, Minneapolis.

chloride, beginning with 2 drops and stopping at 8 drops. Add to each tube 5 drops of the plasma and take time of coagulation (see Table I).

We know by this test that in hemophilias the prothrombin-antithrombin balance is upset, but it is not certainly proved that it is the actual amount of prothrombin that is altered. Addis<sup>1</sup> believes the prothrombin to be present in normal amounts in these cases, but it is so altered as to require longer time for activation to the thrombin present. An excess of antithrombin is present only as a result of decreased prothrombin.

TABLE I

TUBE NO.	1	2	3	4	5	6	7
Calcium chloride	2 drops	3 drops	4 drops	5 drops	6 drops	7 drops	8 drops
Plasma	5 drops	5 drops	5 drops	5 drops	5 drops	5 drops	5 drops
Normal pro-thrombin time	6 min.	8 min.	8 min.	10 min.	10 min.	12 min.	14 min.

The coagulation time of hemophilia is always long. In estimating this time accurately there are many difficulties to consider. The method used is that described by Lee and White.<sup>5</sup> One cubic centimeter of blood is drawn from the vein into a sterile syringe previously washed with normal salt solution, and expressed into a Wassermann tube which has also been rinsed with normal salt solution. Care must be taken to leave salt solution in the needle before puncture as the admission of air bubbles greatly hastens the coagulation of the blood. The needle is removed so as not to break up the platelets in expressing the blood, and the tube is rotated endwise every thirty seconds till the clot adheres to the tube when inverted. Cohen<sup>2</sup> has shown how necessary are absolute cleanliness, uniformity of apparatus, exact amount of blood, and constant temperature.<sup>11</sup> The time of coagulation greatly depends on the extent of contact to the glass; it may be greatly hastened by any shaking of the tube. The hemophilic blood may become gelatinous in a comparatively short time, but it is easily noted that the clot is not firm and that its disturbance results in its breaking down. Minot and Lee<sup>13</sup> found that the clots often formed as quickly as normal but never became as firm. If this first clot is removed, a second clot will form; this is also an imperfect clot and may be removed. It has been observed recently by Lee, but not as yet published, that six and eight clots can be removed in succession from hemophilic blood while that of a normal blood will seldom form a second clot. I was able to remove four clots from the blood in one case, while in normal controls I have never removed more than two.

Case 185593 is worthy of special attention. This patient had a definite history of familial hemophilia; his coagulation time was exceedingly long, but his prothrombin time was within normal limits and his platelet count was 115,000 per c.mm. His mother's blood was then examined and found to have an exceedingly long prothrombin time, though a normal coagulation time. When the prothrombin time was determined, the material was invertible though not as solid and set as in normal sera. Workers in this country have been looking for just such findings, but have not made the actual observations.

Case 182535. In this case there was a coagulation time of 73 minutes, and



after transfusion the coagulation time was reduced to 34 minutes. The prothrombin time was not estimated because of difficulty in procuring blood.

Wright has attributed the hemophiliac condition to a calcium deficiency. Hurwitz and Lucas,<sup>9</sup> in studying five cases in detail, observed the characteristic delay in coagulation and a constant deficiency in prothrombin; other factors of coagulation, however, were present in normal amounts. In the case shown in Table II no appreciable difference in amount or activity of calcium was observed.

TABLE II  
HEMOPHILIA

CASE	PROTHROMBIN TIME (CALCIUM CHLORIDE .5%)							COAGULATION TIME	CALCIUM TIME	PLATELET PER C.MM.
	2 drops	3 drops	4 drops	5 drops	6 drops	7 drops	8 drops			
183593	0	0	12 min.	10.5 min.	10.5 min.	10 min.		93 min.	3 hrs. (imperfect clot)	115,000
	24 hours after transfusion:									
	0	0	75 min.	25 min.	38 min.	39 min.		10 min.	12 min.	171,000
Mother of patient	99 min.	99 min.	Lost	—	—	87 min. (imperfect clot throughout)		8 min.	11.5 min.	202,000

#### EPILEPSY

The results of numerous observations on the blood of patients with epilepsy have been fairly uniform with the exception of the results concerning coagulation time, and these have been most varied. It has been found by several workers to be greatly shortened, while Turner believes the time to be greater in cases of epilepsy. Thorn<sup>14</sup> reports a series of 203 patients with epilepsy whose coagulation time he determined in the method outlined by Lee and White.<sup>10</sup> Ninety-two per cent of these patients fell within normal limits, 5.5 per cent fell under the minimum, and 2.5 per cent were over the maximum. Two and one-half to 14 minutes marked the extremes of the series. Using the same method for determining coagulation time, I studied a series of 9 patients presenting a range of from 4 to 15 minutes, while controls of normal persons ranged from 4 to 12 minutes.

It seems reasonable to conclude that there is no change in the coagulation time of the blood of patients with epilepsy. A great deal depends on the technic, and the differences occurring among normal persons vary in a similar manner.

It is believed that patients with epilepsy may be benefited by calcium lactate. Two patients have returned after a period of from six weeks to two months of the administration of calcium, and were found to have enough calcium in the blood to produce coagulation in less time than by the addition of calcium chloride (Table III).

TABLE III  
EPILEPSY

CASE	PROTHROMBIN TIME (CALCIUM CHLORIDE .5%)								COAGULATION TIME	CALCIUM TIME	PLATELET PER C.MM.
	2 drops	3 drops	4 drops	5 drops	6 drops	7 drops	8 drops				
184,006			Not done					11 min.	Not done	Not done	
185,070			Not done					7 "	5 min.	Not done	
186,346			Not done					6 "	4 "	Not done	
186,105		26 min.	25 min.	26 min.	27 min.			8 "	14 "	Not done	
188,502	0	19 "	15 "	15 "	16 "			12 "	8 "	Not done	
189,332	8 min.	8 "	7 "	7 "	7 "			9 "	10 "	Not done	
191,596	10 "	10 "	10 "	9 "	9 "	9 min.	9 min.	4 "	4 "	178,000	
186,248			Not done					15 "	Not done	Not done	
192,424	6 "	7 "	8 "	8 "	9 "	9 "	9 "	6 "	5 min.	215,000	



into a sterile syringe previously rinsed with salt solution and expressed, without the needle, into a small tube containing 6 drops of a 0.5 per cent calcium chloride solution. The tube is inverted every thirty seconds and the calcium time is taken at the point of coagulation. The test is the same as for coagulation time with the addition of calcium chloride. It has been found that too much as well as too little calcium will serve to delay the process of coagulation.

Blood platelets were isolated in such cases by Lee and Vincent and were found to act normally both in the formation and retraction of the clot.

It has been found experimentally that bile has an inhibitory effect on the formation of thrombin. Bile will entirely prevent coagulation *in vitro* even in the presence of the optimum amount of calcium. It is probable, however, that bile never becomes so concentrated in the blood of jaundiced patients (Tables V and VI).

TABLE VI  
HEMOLYTIC JAUNDICE

CASE	PROTHROMBIN TIME (CALCIUM CHLORIDE .5%)								COAGULATION TIME	CALCIUM TIME	PLATELET PER C.MM.
	2 drops	3 drops	4 drops	5 drops	6 drops	7 drops	8 drops				
192,817	9 min.	9 min.	10 min.	13 min.	14 min.	14 min.	15 min.	14 min.	17 min.	131,000	
190,364	5 "	7 "	7 "	7 "	7 "	9 "	9 "	9 "	5 "	200,000	

#### MYELOGENOUS LEUCEMIA

Little note has been taken in this connection of the blood of myelogenous leucemia. In my series of seven patients it will be seen that there is present a tendency to prolonged prothrombin times, although the coagulation times are quite short. Cohen<sup>2</sup> has shown experimentally that a marked leucocytosis very definitely delays coagulation.

It is evident that these patients have the optimum amount of calcium as the addition *in vitro* of 3 drops of calcium noticeably delays coagulation (Table VII).

TABLE VII  
MYELOCYTIC LEUCEMIA

CASE	PROTHROMBIN TIME (CALCIUM CHLORIDE .5%)								COAGULATION TIME	CALCIUM TIME	PLATELET PER C.MM.
	2 drops	3 drops	4 drops	5 drops	6 drops	7 drops	8 drops				
192,443	7 min.	7 min.	8 min.	9 min.	9 min.	9 min.	10 min.	8 min.	12 min.	196,000	
192,150	16 "	13 "	12 "	12 "	12 "	14 "	14 "	8 "	8 "	194,000	
177,183	Not done							12 "	14 "	270,000	
176,684	17 min.	13 min.	15 min.	2 hrs.	2 hrs.			8 "	9 "	185,000	
186,558	14 "	12 "	13 "	15 min.	15 min.			8 "	11 "	Too many myelocytes	
162,899	Not done							18 "	16 "	280,000	
180,499	15 min.	15 min.	20 min.	20 min.	20 min.	21 min.	25 min.	7 "	6 "	188,000	

#### MISCELLANEOUS CASES

Drinker and Hurwitz<sup>3</sup> found prothrombin to be slightly diminished in all patients with pernicious anemia. I found that to be true in the one patient examined in the Mayo Clinic. The platelet counts are, however, relatively normal.

Three of the splenomegaly cases presented delayed prothrombin times. Coagulation times were rather long, but the addition of calcium tended to retard.

Among this miscellaneous group there were several types of conditions. As control cases they showed nothing abnormal in their factors in coagulation (Table VIII).

TABLE VIII  
MISCELLANEOUS

CASE	PROTHROMBIN TIME (CALCIUM CHLORIDE .5%)							COAGULATION TIME	CALCIUM TIME	PLATELET PER C.MM.
	2 drops 30 min.	3 drops 23 min.	4 drops 16 min.	5 drops 17 min.	6 drops 11 min.	7 drops 15 min.	8 drops			
183,556								4 min.		
Diabetes										
76,654	0	40 "	25 "	25 "	23 "			16 "	22 min.	288,000
Splenic anemia?										
192,553	11 "	13 "	14 "	15 "	15 "	17 "	19 min.	9 "	10 "	177,000
Splenic anemia										
192,503	7 "	9 "	9 "	12 "	14 "	18 "	18 "	10 "	13 "	
Cirrhosis of liver? with splenomegaly										
190,774	9 "	8 "	7 "	7 "	7 "	7 "	8 "	7 "	6 "	197,000
Localized tuberculous splenomegaly										
192,719	14 "	20 "	22 "	24 "	30 "	30 "	30 "	5 "	4 "	204,000
Pernicious anemia										
185,197		Not done						6 "	Not done	157,000
Pernicious anemia										
189,232		Not done						Not done	Not done	269,000
Lues II										
193,565	8 min.	8 min.	7 min.	6 min.	6 min.	6 min.	6 min.	8 min.	9 min.	115,000
Polycythemia										
190,423	8 "	8 "	8 "	7 "	7 "	9 "	9 "	7 "	9 "	208,000
Migraine										
191,091	17 "	17 "	16 "	16 "	16 "	16 "	16 "	15 "	10 "	223,000
Biliary cirrhosis?										
192,121	5 "	5 "	5 "	5 "	5 "	6 "	6 "	7 "	8 "	255,000
Biliary cirrhosis?										
194,103	10 "	12 "	14 "	16 "	15 "	15 "	16 "	9 "	8 "	200,000
Hypernephroma										

#### NOTES ON TECHNIC

In doing these prothrombin tests we have noted that on reducing the amount of blood used to just one-half, the results were identical and the test more economical,—the amount of potassium oxalate being reduced proportionately. Care must be taken to use the same needle for measuring calcium and serum, for the time varies appreciably if the size of the drops is not uniform. I have also done parallel prothrombin tests on several cases, using .5 c.c. of potassium oxalate to 8 c.c. of blood. In the cases of jaundice great difficulty was experienced in avoiding a clot while centrifugalizing the blood. If a clot had formed, the serum it expressed would not clot on the addition of calcium. In the cases in which the serum did not clot while spinning, however, a shorter prothrombin time was manifested when only .5 c.c. of oxalate was used. In these cases there was not sufficient oxalate to precipitate all the calcium, and in one case there was so much native calcium that the optimum amount of calcium brought the shortest time of clotting in the first tube followed by an excess of calcium in the rest of the tubes and a consequent retarding of the clotting-process.

The method employed for platelet enumeration is that described by Wright and Kinnicutt.<sup>16</sup> Blood was mixed with the diluting fluid in the proportion of one to a hundred by means of the ordinary red blood corpuscle pipette and counted in the blood counting chamber with a high dry objective. The diluting fluid consisted of two parts of an aqueous solution of brilliant cresyl blue (1:300) and three parts of an aqueous solution of potassium cyanide (1:1400).



These two solutions must be kept separately and mixed and filtered only as used or a precipitate forms and obscures the platelets. In order to get an even distribution of platelets, it is well to fill the chamber at once after mixing blood and fluid in the pipette.

I found that waiting an appreciable length of time and shaking as for red blood corpuscles and white blood corpuscles is not satisfactory. Wright<sup>17</sup> demonstrated several years ago that platelets tend to adhere to any foreign body and form clumps. This perhaps explains the reduced counts after shaking the pipette. Shaking also tends to form larger and denser clumps which makes the count very inaccurate. There are three main requisites for reliable platelet counts; namely, the red corpuscles must be laked, the protoplasm of the leucocytes well stained, and the platelets evenly distributed.<sup>4</sup>

After the counting chamber has been filled, it may stand for hours before making the count. It must surely stand ten minutes to allow the platelets thoroughly to settle. The cresyl blue solution will keep indefinitely on ice, which prevents growth of yeasts. Potassium cyanide should be made up fresh at least every ten days. It must be made of pure potassium cyanide not undergoing degeneration.

Duke found platelet counts fairly constant in normal persons but fluctuating to extremes in pathologic conditions. By various experimental devices he showed that the platelet count fluctuated in exact proportion to the degree of toxicity. He found that the injection of diphtheria toxin in small doses raised the count and that the same toxin in lethal or nearly lethal doses decreased the count rapidly. He concluded from his experiments that when increased counts were associated with pathologic conditions the case was usually mild. In other words, the toxins act as irritants or poisons according to the size of dosage. Duke<sup>6</sup> bore out his deductions in man; his average counts of patients in febrile condition was 114,000 per c.mm.—the convalescent counts going as high as 750,000 per c.mm.

#### SUMMARY

1. The technic of the various laboratory methods used is described.
2. There was a marked calcium deficiency in jaundice cases of several weeks' duration. Platelets were present in normal numbers.
3. The blood of hemorrhagic purpuras was deficient in platelets. There was no retraction of the clot. Estimation of platelets was of value in differentiating between hemorrhagic purpuras and hemophilias.
4. The coagulation time of epileptic blood was within normal limits.
5. Prothrombin was slightly diminished in pernicious anemia.
6. There was a tendency in myelocytic leucemias for prothrombin time to be prolonged beyond the normal limits of from six to fourteen minutes, although the coagulation time was within normal limits.
7. There was a characteristic delay in coagulation in the blood of hemophiliac cases. There was also a deficiency in prothrombin. Platelets were present in normal numbers and have normal retractile powers. There was apparently no deficiency in calcium.
8. One patient of special note among the hemophilias had an extremely

long coagulation time though his prothrombin time was within normal limits. His mother had a normal coagulation time but a markedly delayed prothrombin time.

## BIBLIOGRAPHY

- <sup>1</sup>Addis, T.: The Pathogenesis of Hereditary Hemophilia, *Jour. Path. and Bacteriol.*, 1911, xv, 427-452.
- <sup>2</sup>Cohen, M. S.: The Coagulation Time of the Blood as Affected by Various Conditions, *Arch. Int. Med.*, 1911, viii, 684-716.
- <sup>3</sup>Drinker, C. K., and Hurwitz, S. H.: The Factors of Coagulation in Primary Pernicious Anemia, *Arch. Int. Med.*, 1915, xv, 733-745.
- <sup>4</sup>Duke, W. W.: The Rate of Regeneration of Blood Platelets, *Jour. Exper. Med.*, 1911, xiv, 265-273.
- <sup>5</sup>Duke, W. W.: The Pathogenesis of Purpura Hemorrhagica with Especial Reference to the Part Played by Blood Platelets, *Arch. Int. Med.*, 1912, x, 445-469.
- <sup>6</sup>Duke, W. W.: Causes of Variation in the Platelet Count; Experimental Results Showing the Effect of Diphtheria Toxin, Benzol and Tuberculin on the Platelet Count in Rabbits, *Arch. Int. Med.*, 1913, xi, 100-120.
- <sup>7</sup>Howell, W. H.: The Role of Antithrombin and Thromboplastin (Thromboplastic Substance) in the Coagulation of Blood, *Am. Jour. Physiol.*, 1911, xxix, 187-209.
- <sup>8</sup>Howell, W. H.: The Condition of the Blood in Hemophilia Thrombosis and Purpura, *Arch. Int. Med.*, 1914, xiii, 76-95.
- <sup>9</sup>Hurwitz, S. H., and Lucas, W. P.: A Study of the Blood in Hemophilia, *Arch. Int. Med.*, 1916, xvii, 543-569.
- <sup>10</sup>Lee, R. J., and White, P. D.: A Clinical Study of the Coagulation Time of Blood, *Am. Jour. Med. Sc.*, 1913, cxlv, 495-503.
- <sup>11</sup>Lee, R. J., and Vincent, B.: The Relation of Calcium to the Delayed Coagulation of Blood in Obstructive Jaundice, *Arch. Int. Med.*, 1915, xvi, 59-66.
- <sup>12</sup>Minot, G. R.: The Effect of Temperature upon the Clotting Time (Prothrombin Time) of Oxalated Plasma with Calcium, *Jour. Med. Research*, 1915-16, xxxiii, 503-506.
- <sup>13</sup>Minot, G. R., and Lee, R. J.: The Blood Platelets in Hemophilia, *Arch. Int. Med.*, 1916, xviii, 474-495.
- <sup>14</sup>Thorn, D. A.: Coagulation Time of the Blood in Epileptics, *Ill. Med. Jour.*, 1914, xxvi, 382, 383.
- <sup>15</sup>Wright, A. E.: On a Method of Determining the Condition of Blood Coagulability for Clinical and Experimental Purposes, and on the Effect of the Administration of Calcium Salts in Haemophilia and Actual or Threatened Hemorrhage, *Brit. Med. Jour.*, 1893, ii, 223-225.
- <sup>16</sup>Wright, J. H., and Kinnicutt, R.: A New Method of Counting the Blood Platelets for Clinical Purposes, and some of the Results Obtained with It, *Jour. Am. Med. Assn.*, 1911, lvi, 1457-1459.
- <sup>17</sup>Wright, J. H.: The Histogenesis of the Blood Platelets, *Jour. Morphology*, 1910, xxi, 263-278.

## CHRONIC TONSIL INFECTIONS\*

BY JOSIAH J. MOORE, M.S., M.D., CHICAGO, ILL.

AS an introduction to the discussion of the pathology of the tonsils, the following remarks on their anatomy would seem pertinent.

The faucial tonsils are two symmetrically placed prominent bodies situated between the anterior and posterior pillars of the soft palate and covered by a layer of epithelium which is continuous with the mouth cavity. The prominence of the bodies varies at different ages and in individuals of the same age. MacLachlan in his series gives the average size as 2.5x1.8x1.5 cm. In 250 pairs of tonsils examined in a routine manner I found the average size to be somewhat smaller or 2.3x1.5x1.0 cm.

Each tonsil is enclosed in a fibrous capsule which surrounds it completely except on the inner surface where the openings on the mucosal surface are found. The mucous surface covering the tonsil varies considerably in appearance. It is usually convex. Depending upon the character of the mouths of the crypts it may be either ragged or smooth.

The crypts of the tonsil are diverticula of the buccal cavity lined by squamous epithelium. They are ten to fifteen in number extending into the tonsil almost to the capsule where they end blindly. The contents of the crypt are saliva, food debris, cell debris and bacteria, the contents of the mouth cavity proper. Branching from the main crypts are numerous smaller crypts which extend for a considerable distance into the tonsillar substance.

The tonsil substance proper is composed of trabeculae, stroma, follicles, blood and lymph vessels and the individual cells of the tonsils.

The trabeculae are fibrous bands arising from the capsule which extend across the tonsil becoming smaller toward the buccal surface. They carry large vessels and lymphatics, and give partial origin to the stroma of the tonsil. The vessels of the trabeculae are quite large. The stroma is a loose connective tissue structure forming the supporting framework for the tonsil cells.

The follicles make up the greater part of the tonsil substance proper. They consist of numerous collections of cells, similar in structure to the follicles of the lymph nodes. The central cells, constituting about one-third of the follicle, are large with prominent nuclei and abundant cytoplasm. At the periphery the cells are smaller with deeper staining nuclei and less protoplasm. The inter-follicular tissue is made up of a very loose reticular stroma containing many fine blood and lymph capillaries and packed with small mononuclear cells. Plasma cells are constantly found in the tonsil from the second week after birth.

The blood vessels and lymphatics of the tonsils follow the trabeculae are similar to those of any other organ.<sup>†</sup>

\*From the Department of Pathology and Bacteriology, University of Illinois, College of Medicine, Chicago, Ill.

†An excellent discussion of the anatomy, physiology and other phases of this subject has been given by MacLachlan: Tonsillitis, University of Pittsburg, School of Medicine, Pub., 1912.

Due both to its position and structure the tonsil is more prone to inflammation than almost any other organ in the body. Owing to the former it comes in direct contact with the various species of bacteria in the mouth cavity. More important, however, is the deposition of these bacteria with food and other debris in the lumina of the crypts. Here sluggish drainage, ideal temperature and moisture conditions, and varying degrees of oxygen pressure furnish the organisms with a splendid opportunity for development. Depending upon the type and virulence of the organism and the resistance of the host, either an acute or a chronic condition results. Although the majority of tonsillar inflammatory conditions arise from irritants reaching the organ from the buccal cavity, some, such as secondary syphilis, are metastatic in nature.

In this discussion on chronic tonsil infections the classification used is based, not upon clinical data, but upon actual changes in the tissue recognized pathologically as chronic inflammation. Various classifications have been suggested, but the following by Semon and Williams<sup>1</sup>, based upon the location of the lesion, is at least convenient. (a) Chronic lacunar or cryptic tonsillitis; (b) chronic interstitial tonsillitis; and (c) chronic peritonsillitis. These types correspond with a similar classification of acute conditions to which they are closely related. Either one may precede the other, or they may and frequently do exist simultaneously.

*Chronic Lacunar Tonsillitis.*—Chronic lacunar tonsillitis is the most common lesion. Changes are found in the lining, size, and contents of the crypts. In chronic lacunar inflammations the epithelium is irregular due to the dilatation of the finer branches of the main crypts, papillomatous masses of keratinized cells, and desquamation of cells. The cavity of the crypts is usually dilated, containing desquamated epithelial cells, cholesterol crystals, polymorphonuclear cells, hyaline material and bacteria. Mycelial growths, or "actinomyces-like bodies," are often found in this condition. The shape of the dilated crypts and the compressed epithelial lining suggests blocking of the mouths.

It is in this type of tonsillitis that the soft, yellowish, foul-smelling, cheese-like particles may be frequently squeezed from the crypts. Not infrequently calcium salts are deposited in such masses, forming tonsilloliths. Occasionally there is a plugging of one of the crypts with an increase in the keratinized epithelial cells, forming an epithelial cyst.

*Chronic Interstitial Tonsillitis.*—Chronic interstitial tonsillitis is characterized by an increase in connective tissue. Since this is derived from the capsule and trabeculae, there may be an increase in the size of the trabeculae or an increase in the amount of stroma in the parenchyma of the organ. These two forms are usually associated. In small hidden atrophic tonsils fibrosis is to be expected, but it is often found in hypertrophied tonsils. It is generally conceded, however, that the hypertrophic finally passes into the atrophic state. Islands of cartilage or bone occasionally appear in the stroma in fibrosis of the tonsils. Here also we see the thick-walled vessels which so often cause hemorrhage in tonsillectomy in this type of inflammation. Chronic lacunar and interstitial tonsillitis are often found together. In 250 pairs of tonsils examined they were found associated in 42 per cent.



Closely associated with these types of tonsillitis is the condition termed hypertrophy of the tonsil. The etiologic factors are numerous. Heredity is thought by some to play an important role. Constitutional or predisposing causes are tuberculosis, syphilis, gout and alcoholism in parents. Local causes, such as recurrent attacks of tonsillitis, are, however, most important. Usually the only distinctive point clinically in the gross appearance of the tonsil is its actual size. Our largest tonsil measured 4x3x1.7 cm.

The most common microscopic picture in the hypertrophy of the tonsil on children is an increase in both size and number of follicles so that they occupy more space than the diffuse lymphoid tissue. The enlargement is in all probability due to hyperplasia of the cells in the center of the follicles. In adults the follicles of the hypertrophied tonsil are usually diffuse and lymphoid tissue with stroma make up the major portion of the tonsil. Practically always some form of inflammation may be found if properly sought. Most commonly this is acute or chronic lacunar tonsillitis. Hyperplasia of the lymphoid tissue was observed in 30 per cent of the series examined.

Plasma cells in large numbers are found in all hypertrophied tonsils, and since these cells are usually indicative of chronic infection, their presence suggests this as a possible etiologic feature in the hypertrophy. Plasma cells are found, however, in abundance in all tonsils, normal or pathologic, though more numerous in the latter.

It is sometimes difficult to differentiate between a fibrotic atrophic tonsil the result of chronic inflammation and one undergoing the normal atrophy. Features of diagnostic importance would be, clinically, the age of the individual and the history of repeated attacks of acute tonsillitis, and microscopically the size and contents of the crypts and the presence of lacunar inflammation.

*Chronic Peritonsillitis.*—Chronic peritonsillitis is characterized by an increase in the fibrous tissue in the capsule and pericapsular tissue with infiltration of characteristic inflammatory cells. Cartilage and bone are occasionally found in the thickened capsule.

Of special interest as chronic infections of the tonsil, are those of tuberculosis and syphilis. It is questionable whether a diagnosis of primary tuberculosis of the tonsil can be made during life. Statistics on the frequency of primary and secondary tuberculosis of the tonsils may be very misleading even if accompanied by data indicating the presence or absence of tuberculosis in other parts of the body. The frequency of tuberculosis when no other foci are demonstrable varies from 1 to 5 per cent. In patients suffering from pulmonary tuberculosis the tonsils are affected frequently. Wood<sup>2</sup> believes that 90 per cent of tuberculous cervical adenitis is secondary to tuberculous infection of the tonsil. It has been demonstrated, however, that the tubercle bacillus can pass through the tonsil and to the lymph glands, with no apparent injury to the tonsil. Ravenel,<sup>3</sup> by feeding tubercle bacilli to hogs, found that the bacilli passed through the intestinal mucosa to the chyle and mesenteric lymph glands without producing microscopic lesions in the intestines.

In my series of 250 pairs of tonsils, tuberculosis was present in 2.5 per cent. Clinical examination failed to disclose involvement of either the lungs or the

cervical lymph glands. The ages ranged from 6 to 25 years of age. The presence of typical tubercles on microscopic sections was considered sufficient for diagnosis. They revealed caseation, epithelial cells, giant cells, and connective tissue changes. Giant cells alone might be due to a variety of causes, as foreign bodies, cholesterin crystals, and syphilis.

Syphilis of the tonsil may be primary, secondary, or tertiary. According to some the tonsil is more generally involved in extragenital primary lesions because its structure gives greater opportunity for the spirochete to lodge. The percentage varies from 6 to 55 per cent. Women are most frequently affected. The lesion occupies the greater part of the mucous surface, with sharply defined margins and considerable induration. They are usually painless. Enlargement of the lymph glands under the angle of the jaw is always observed.

Secondary syphilis shows itself in the tonsil in the form of mucous patches, occasionally by superficial ulceration.

Tertiary lesions are the gummata or the resulting ulcerations. They are rare. Healing of the gummata results in the formation of much scar tissue with marked induration of the tonsil.

Actinomycosis of the tonsil has been occasionally described. Davis<sup>4</sup>, from his own work and from a review of the literature, thinks that no well-authenticated case of primary actinomycosis of the tonsil has been reported. The "actinomyces-like bodies" or granules found in the crypts are not true actinomyces, but consist of streptococci, bacilli, and spirilla growing together in a more or less symbiotic relationship. The bacilli belong to the fusiform group and neither they nor the streptococci have any marked pathogenicity for animals. These granules are in about one out of every three or four pairs of tonsils examined. Probably all tonsils at times contain them.

The bacteriologic flora of the tonsil is somewhat similar in both acute and chronic tonsillitis. The surface bacteria are usually those found upon all surfaces of the buccal cavity and come from the throat, mouth, nose, food and inspired air. Cultures from the surface are of little value in determining the type of organism in the crypts. In the crypts, many varieties of microorganisms have been found, but those predominating are streptococci. Davis<sup>5</sup>, isolated hemolytic streptococci in the majority of his series of 133 tonsils, with pneumococcus, staphylococcus, *B. diptheriæ*, *B. influenzae* and *B. mucosus capsulatus* in a small number of cases. These streptococci are capable of producing arthritis in rabbits. They were isolated from individuals suffering from chronic arthritis, endocarditis, and nephritis, but it is not certain that they were causative factors in all cases. It must be recognized that in almost all tonsils, whether pathologic or not, hemolytic streptococci capable of causing arthritis in rabbits may be isolated from the crypts.

Frequent favorable results following removal of infected tonsils suggest that the absorption of bacteria or their products from the crypts has much to do with the causation of certain chronic lesions. The severity and site of these lesions may depend upon the virulence and specificity of the bacteria and the general and local resistance of the host. Rosenow<sup>6</sup>, suggests that certain organisms have an elective affinity for different tissues. Strains of streptococci ob-

tained from appendicitis, ulcer of the stomach, cholecystitis, rheumatic fever, erythema nodosum, myocarditis, endocarditis, and herpes zoster when injected intravenously into animals, rabbits and dogs, elect to produce the specific lesion from which they were isolated in a very high percentage of cases. Not only do strains isolated from the specific lesions, but also strains isolated from the apparent focus of infection, which frequently was the tonsil, give more or less comparable results. He likens a pocket in the tonsils filled with pus, necrotic material, and bacteria to a culture tube having a permeable wall affording abundant opportunity for the entrance of bacteria and their products. The importance of such foci of infection to systemic disease is especially emphasized by Billings<sup>7</sup> in his extensive study.

In a series of rabbits studied, using an organism isolated from a patient with subacute arthritis, endocarditis occurred twice, nephritis three times, and arthritis in all. In a series of forty-five rabbits injected with a hemolytic streptococcus isolated from the tonsillar crypts during an attack of acute tonsillitis in a patient having chronic arthritis, arthritis developed in all the rabbits, endocarditis in 50 per cent, kidney lesions in 15 per cent, intestinal hemorrhage 6.6 per cent, hemorrhage in the stomach in 5 per cent, appendicitis and gall bladder lesions 2.5 per cent, and myocarditis in nearly all.<sup>8</sup>

It is interesting to compare this last group with a group recently injected with a hemolytic streptococcus from a case of acute tonsillitis with no complications. Fourteen rabbits were injected. The percentage of lesions was as follows: arthritis 90 per cent, endocarditis 50 per cent, nephritis 28 per cent, myositis 42 per cent, myocarditis 21 per cent, adenitis 14 per cent and hemorrhages into the gall bladder 17 per cent. The variable number of lesions produced by these different strains possibly illustrates Rosenow's hypothesis that "the tendency to locate electively within a limited range, monotropism, is most highly developed in the nonvirulent strains isolated from chronic lesions." In the more virulent strains from acute lesions this tendency is less highly developed, the lesions occurring over a wider range, "polytropism."

Reference has already been made to the organisms composing the so-called "actinomyces-like bodies" found in the crypts. Pilot and Davis<sup>9</sup>, have made anaerobic cultures of many granules found in tonsils and frequently obtained hemolytic and nonhemolytic streptococci of low virulence capable in large doses of producing arthritis in rabbits. On early transplants they grow better under anaerobic conditions than under aerobic conditions. Later they lose this characteristic.

The tubercle bacillus is rarely found in the tonsillar crypts. Occasionally diphtheria and influenza bacilli<sup>10</sup> occur here, where they may persist for a long time. Diphtheroids are not uncommon in the crypts.

The persistence of virulent diphtheria bacilli in the crypts of the tonsils brings up the very important problem of the role played by these organs in disease "carriers." Much has been written on this subject and reference to some of the more recent literature will demonstrate its importance. Friedberg<sup>11</sup> found that persistent diphtheria carriers became negative after removal of the tonsils. In microscopic sections of these tonsils<sup>12</sup> the diphtheria bacilli were seen in

superficial ulcers located usually in the crypts. In nineteen diphtheria carriers Ruh, Miller and Perkins<sup>13</sup> isolated pure cultures of *B. diphtheriae* from the tonsillar crypts both before and after tonsillectomy. All these writers recommend the removal of the tonsils in individuals where diphtheria bacilli persist for any considerable period of time.

It is of interest that the crypts of the tonsils may become chronically infected with meningococci. Although in meningococci carriers the organisms are usually isolated from the nasopharynx, Mathers<sup>14</sup> recently has obtained them in several cases from the crypts of extirpated tonsils. Whether removal of the tonsils will free the carriers of meningococci is still a matter of conjecture.

A third disease where the virus may be harbored in the tonsil is poliomyelitis. Rosenow<sup>15</sup> and his coworkers and Nuzum<sup>16</sup> have obtained organisms from the crypts of tonsils in patients with or recovering from poliomyelitis which resemble morphologically and culturally, and produce the same type of paralysis in animals, as the cocci which they isolate from the spinal cord of fatal cases. Sydell<sup>17</sup> concludes that the tonsils and adenoids may be the portals of infection for poliomyelitis. Their experiments suggest that the tonsils may act as the resting place for the virus. Flexner<sup>18</sup> states more conclusively that "the virus of the poliomyelitis has been traced to the respiratory mucous membrane of healthy persons who may act as carriers and has been found in several instances in the tonsils removed by operation several months after recovery from the acute disease, so that the existence of so-called chronic carriers has also been indicated."

In a general way these examples suggest that the infectious organism or virus of many diseases which have as their portals of entry the upper respiratory system, may lodge either permanently, or for a long period in the crypts of the tonsils, thus producing the state which we term a "chronic carrier." The removal of the infected tonsil may aid materially in controlling such diseases.

#### BIBLIOGRAPHY

- <sup>1</sup>Allbutt-Rolleston: *System of Medicine*, iv, Part 3, 174.
- <sup>2</sup>Wood: *Jour. Am. Med. Assn.*, 1905, xlv, 1425.
- <sup>3</sup>Ravenel: *Jour. Med. Research*, 1903, x, 460.
- <sup>4</sup>Davis: *Jour. Infect. Dis.*, 1914, xiv, 144.
- <sup>5</sup>Davis: *Jour. Infect. Dis.*, 1912, x, 148.
- <sup>6</sup>Rosenow: *Jour. Am. Med. Assn.*, 1915, lxv, 1687.
- <sup>7</sup>Billings: *Focal Infection*, D. Appleton & Co., 1916.
- <sup>8</sup>Jackson: *Jour. Infect. Dis.*, 1912, xi, 243.
- <sup>9</sup>Pilot and Davis: *Personal communication*.
- <sup>10</sup>*Jour. Am. Med. Assn.*, 1915, lxiv, 1814.
- <sup>11</sup>Friedberg: *Jour. Am. Med. Assn.*, 1916, lxvi, 810.
- <sup>12</sup>Rappaport: *Jour. Am. Med. Assn.*, 1916, lxvi, 943.
- <sup>13</sup>Ruh, Miller and Perkins: *Jour. Am. Med. Assn.*, 1916, lxvi, 941.
- <sup>14</sup>Mathers: *Personal communication*.
- <sup>15</sup>Rosenow: *Jour. Am. Med. Assn.*, 1916, lxvii, 1202.
- <sup>16</sup>Nuzum: *Jour. Am. Med. Assn.*, 1916, lxvii, 1205.
- <sup>17</sup>Sydell: *Ann. Otol., Rhin., and Laryng.*, 1917, xxvi, 98.
- <sup>18</sup>Flexner: *Am. Jour., Med., Sc.*, 1917, cliii, 157.



## LABORATORY METHODS

---

### A RAPID COLORIMETRIC METHOD FOR ESTIMATING GLUCOSE IN URINE\*

---

BY VICTOR I. ISAACSON, B.S., NEW YORK CITY

SEVERAL excellent methods are at present in use for quantitatively determining glucose in urine. The procedure described below is reported, however, because it is simple as well as accurate and requires little skill and time. The new method, like many of the older ones, depends upon the fact that alkaline copper sulphate solutions are reduced by glucose at higher temperatures. The quantity of copper sulphate reduced is computed indirectly by determining the amount left unreduced. This latter portion is measured in a colorimeter<sup>†</sup> by comparing the intensity of its blue ammonia compound with a standard solution of the same substance.

*Solutions.*—The solutions used are: (1) Concentrated ammonia water. (2) A solution resembling Benedict's qualitative sugar reagent, containing 34.65 gm. copper sulphate with five molecules of water, 100 gm. anhydrous sodium carbonate and 100 gm. sodium citrate in one liter of water. The copper sulphate should be dissolved separately in about 200 c.c. of water and then added to the solution of the other two reagents with stirring. The whole is made up to one liter. Ten c.c. of this solution is equivalent to 50 mg. of glucose.

*Standards.*—Standard A is made by measuring 10 c.c. of the solution just described into a 25 c.c. volumetric flask, adding 3 c.c. of concentrated ammonia water, diluting to the mark, and shaking until uniformity of intensity is insured. The numbers appearing to the left of the calibrations on the graduated tube (Fig. 2) give the result directly in per cent when working with this standard.

Standard B is half as strong and is prepared by measuring 5 c.c. of the reagent into a 25 c.c. flask, adding 3 c.c. of concentrated ammonia, diluting to the mark, and shaking. When using this standard for comparison, the numbers in the right-hand column on the calibrated tube yield the answer directly in per cent.

Standard C is intermediate in intensity between A and B and is made by mixing equal portions of these two standards. The results for this standard do not appear on the calibrated tube, but may be secured by dividing the sum of the two numbers at any given point on the tube by two. In every case these figures give the quantity of glucose in the specimen *directly in per cent* provided only 1 c.c. of the specimen was used for analysis. For other quantities the figures read off must be divided by the number of cubic centimeters of urine employed. The standard color solutions are transferred to the ungraduated

\*From the Chemical Laboratory of Montefiore Home and Hospital, New York City.

†The apparatus may be obtained from Eimer & Amend, New York City.

tubes, labeled A, B, and C, stoppered tightly, and kept preferably in a cool place.

*Technic.*—Ten c.c. of the copper sulphate solution is measured into a  $7 \times \frac{7}{8}$  inch test tube. Between 10 and 30 mg. of glucose is the most favorable quantity with which to work. If the qualitative test has indicated 1 per cent or more of glucose, 1 c.c. of the specimen is added. For percentages less than one, it is advisable to use 3 c.c. of urine; and for percentages approaching 3.5, it is necessary to reduce the quantity used to  $\frac{1}{2}$  c.c., to make the precipitation quantitative. Two glass beads are added to prevent bumping, the test tube is shaken and the contents *boiled* for three minutes over the free flame. About one-tenth of a gram of animal charcoal is added, and the solution is filtered through a folded filter paper into the graduated tube, allowing the first 10 to 15 drops to flow back into the test tube. When all is filtered, the paper is washed with a few drops of water repeatedly until the filtrate is colorless. Three c.c. of concentrated ammonia



Fig. 1.

Fig. 1.—Sketch of colorimeter, color tubes, and apparatus required in the procedure.

Fig. 2.—Comparison tube. Numbers in the left-hand column are read when working with darkest standard. Those in right-hand column for the lightest. The average of two figures on same line gives answer for intermediate standard.

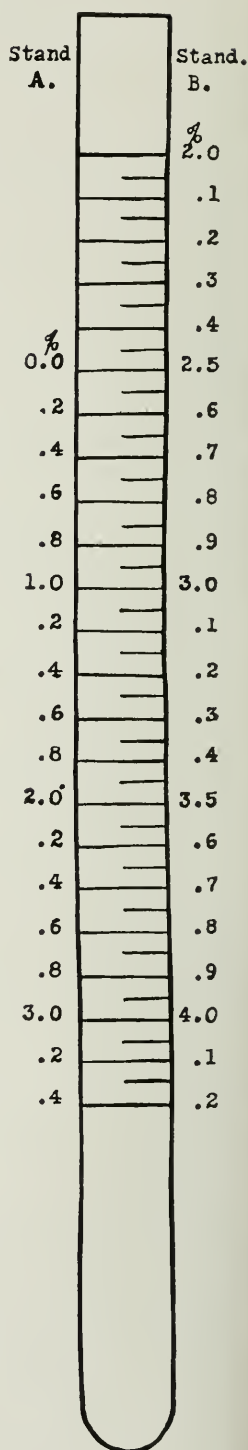


Fig. 2.

water are added to the contents of the tube to develop the color; the tube is inverted to insure mixing, and water is added from a medicine dropper until the intensity is the same as that of one of the three standards. The reading taken will depend on the standard used.

Although there are no steps that offer any difficulty, a few suggestions are necessary. The amount of citrate in solution influences the intensity of the blue color; the same quantity within one gram should, therefore, be weighed out each time more of the reagent is prepared. One should make no attempt to reduce the time of boiling else the precipitation may not be complete. Care should be taken to refilter the first portion that comes through, since the cloudiness is due to cuprous hydroxide which dissolves in ammonia and yields on oxidation a blue color identical with that formed by cupric salts and ammonia. No trouble is encountered in most cases. When the turbidity persists, several times the quantity of animal charcoal ordinarily used should be added. One should avoid the use of too much wash water since this may make the filtrate of concentrated sugar solutions too light to read. The ammonia water need not be measured accurately. Foaming may be prevented by adding two or three drops of caprylic alcohol.\*

The operations may be concisely summarized as follows:

1. Pipette 10 c.c. of the reagent into a test tube.
2. Add  $\frac{1}{2}$ , 1, or 3 c.c. of urine, depending on concentration.
3. Drop in 2 glass beads, shake, and *boil for three minutes*.
4. Add about  $\frac{1}{10}$  gram of animal charcoal and filter, refiltering the first small portion.
5. Add about 3 c.c. of concentrated ammonia water to the filtrate, invert several times and compare.
6. Read the proper figure, and if a quantity other than 1 c.c. of urine was used, divide the figure by the number of cubic centimeters used.

A few time-saving devices have been worked out for use when *several* analyses have to be done at the same time. A beaker of 750 c.c. capacity half filled with water may be used as a heating agent. When the reagent and specimens have been measured, the test tubes are inserted and allowed to stand in the beaker for four to five minutes *from the time the water has begun to boil vigorously*. Bumping can be prevented by introducing into the bottom of the beaker a piece of wire gauze of half inch mesh turned down at the sides. The remaining steps are the same as described above. Filtrations and washings can be performed simultaneously.

A method similar to the one just described was proposed by Autenrieth and Tesdorpf† in 1910 and later modified by Eschle.‡ They proposed the colorimetric measurement of the unreduced portion of a Bang's copper sulfate solution. The quantities used, however, were large (60 c.c.), the reagents costly, and moreover unstable, autoreduction taking place on boiling the solution. Considerable time had to be expended in standardizing the apparatus. Ammonia could not be used to develop the blue color and the faint green color of the copper sulphate

\*Specimens which contain albumin must first be heated and filtered, since the protein modifies the blue color to such an extent as to make comparison difficult.

†Autenrieth, W., and Tesdorpf, Th.: München. med. Wchnschr., 1910, lvii, 1780.

‡Eschle, O.: Fortschr. d. Med., 1912, ii, 326.

was not well adapted for comparisons. These difficulties are overcome in the new procedure and a further decided advantage is the use of the same reagent as a qualitative and quantitative solution.

The results of comparative analyses made with the new method and by the Pavy-Kumagawa procedure are recorded in Table I. Analyses of pure glucose solutions further indicate that the new procedure is accurate. A 1 per cent solution gave 1.04 per cent; a 2 per cent solution, 2.04 per cent, 2.08 per cent; and a 3 per cent solution, 3.07 per cent, 3.05 per cent, 3.11 per cent. Above 3 per cent the precipitation is not quantitative and, therefore, it is essential to employ only  $\frac{1}{2}$  c.c. of the specimen for such concentrations.

The same solution may be employed as a qualitative reagent. Either 5 c.c. of the reagent and 10 drops of urine, or 10 c.c. of the reagent and 1 c.c. of the specimen should be used to make the test. The mixture should be boiled two minutes over the free flame or left in a beaker of boiling water for five minutes. The use of 10 c.c. of the reagent and 1 c.c. of the specimen, both accurately measured, is advisable if a quantitative procedure is to follow, since measurements need not be repeated. The reagent gives an easily discernible turbidity with a 0.05 per cent pure glucose solution. The results of comparative qualitative tests made with this and with Benedict's qualitative sugar reagent are summarized below. It is evident that its sensitiveness as a qualitative solution enhances the value of the quantitative procedure.

A. Ten c.c. new reagent and 1 c.c. of 0.10 per cent pure glucose solution, marked cloudiness throughout solution in less than a half minute boiling. Five c.c. Benedict's reagent and 1 c.c. same glucose solution, slight reddish precipitate after three minutes boiling but no general cloudiness. Repeated with 9 drops of glucose solution and 5 c.c. Benedict's, boiled three minutes, slight red precipitate.

B. Ten c.c. new reagent and 1 c.c. 0.05 per cent pure glucose solution, one-half minute after boiling commences, turbidity is evident throughout the solution. Five c.c. Benedict's reagent and 1 c.c. of the same glucose solution, several specks of cuprous oxide float about, but they might be easily overlooked.

The following comparative tests were made on urines of diabetic patients. All were tested by using 1 c.c. of the specimen, 10 c.c. of the new reagent and kept in a steam bath for 5 minutes from the time boiling commenced. The quantities used for the test according to Benedict were those recommended by that author.

(a) G. Solution remains clear	Benedict negative.
(b) B. Solution remains clear	Benedict negative.
(c) P. Slight yellow precipitate on standing	Benedict negative.
(d) M. Very slight yellow precipitate settles out on standing	Benedict negative.
(e) P. Solution remains clear	Benedict negative.
(f) G. Considerable green precipitate throughout indicating trace	Benedict trace.
(g) S. Heavy green precipitate after 30 seconds, turning yellow on further boiling	Benedict similar.

These tests indicate that in no case where a negative result was obtained with the new reagent, did Benedict's reagent give a positive reaction. In cer-



tain instances of diabetic specimens where Benedict's gave a negative result, slight traces were discovered by the use of the modified reagent. Test made on urinary specimens of normal individuals yielded negative results in every case.

An explanation of the manner in which the figures on the graduated tube are arrived at is now presented. The graduated tube (Fig. 2) has been *divided* into tenths of a cubic centimeter, but in order to save calculation, the figures have been made to *read off* the answer directly in per cent, provided *one c.c.* of the specimen is used for analysis. The manner in which these numbers are obtained is as follows:

$$\% = \frac{\text{mg. of glucose present}}{\text{wt. of specimen in mg.}} \text{ times } 100 \quad (1)$$

The numerator of this fraction is equal to the amount of  $\text{CuSO}_4$  started with, less the amount found unreduced, both expressed in terms of mg. of glucose. The amount started with is always the same and is equal to 50 mg. of glucose. The amount left unreduced is equivalent to the number of c.c. to which the filtrate has to be diluted in order to be of the same intensity as that of the standard with which it is being compared, multiplied by the value in mg. of glucose of 1 c.c. of that standard.

The denominator will be 1000, assuming 1 c.c. of urine is used. For other quantities this number will be different.

The right-hand side of the equation therefore becomes

$$\frac{50 - (\text{number of c.c., times the value of 1 c.c.})}{10} \quad (2)$$

Putting for the value of 1 c.c. of the standard the letters  $K_A$   $K_B$   $K_C$  for standards A, B and C, respectively, we may write instead of equation 2,

$$\frac{50 - (\text{number of c.c., times } K_A, K_B, \text{ or } K_C)}{10} \quad (3)$$

and since Standard A contains copper sulphate equivalent to 50 mg. of glucose in 25 c.c., 1 c.c. is equivalent to 2 mg. Similarly  $K_B$  is 1 and  $K_C$  is  $1\frac{1}{2}$ .

If the unreduced copper sulphate had to be diluted, for example, to 20 c.c. in order to match Standard A, then the answer in per cent is according to equation 3,

$$\frac{50 - (20 \text{ times } 2)}{10} = 1.0$$

and 1 per cent, therefore, appears where the tube measures 20 c.c. If Standard B were being used for comparison, the  $K_B$  being 1, the equation would read

$$\frac{50 - (20 \text{ times } 1)}{10} = 3.0$$

We find, therefore, in the right-hand column where the figures for Standard B appear, the figure 3.0 at the point where the tube measures 20 c.c.

Since the tube measures 25 c.c. at the point marked 0.0% it may be substituted for a 25 c.c. volumetric flask in making up the standards.

TABLE SHOWING RESULTS OF COMPARATIVE ANALYSES

AUTHOR'S METHOD	PAVY- KUMAGAWA METHOD	AUTHOR'S METHOD	PAVY- KUMAGAWA METHOD	AUTHOR'S METHOD	PAVY- KUMAGAWA METHOD
2.79		0.40	0.50	0.92	
2.72	2.76	1.76	1.84	0.92	0.93
1.48				1.04	
1.44	1.27	0.62	0.58	1.14	1.06
0.48		8.40	8.57	1.34	
0.45	0.49	0.88	0.72	1.40	1.47
1.56				0.40	
1.52	1.45	1.20	1.32	0.44	0.53
0.72		1.14	1.27	1.36	
0.74	0.67	1.12	1.09	1.34	1.27
0.98				0.20	
1.00	0.86	6.70	6.70	0.27	0.32
0.22		3.30	3.30	0.36	
0.21	0.23			0.40	0.33
		2.10	2.12		
1.20		1.62	1.64	0.47	
1.20	1.14			0.53	0.47
0.48		1.94	2.17	0.24	
0.48	0.45	0.20	0.28	0.26	0.24
0.22		0.44	0.52	2.10	2.00*
0.21	0.23			2.00	2.08
0.57		0.44	0.53	1.08	
0.60	0.57	1.14	1.17	1.02	1.03*
0.23				1.36	
0.19	0.20	5.5	5.8	1.44	1.35*
		2.61	2.46		
0.22		1.00	1.08	1.92	1.88
0.22	0.21	0.29	0.35	0.18	0.25
1.80		1.26	1.27	1.80	1.88
1.78	1.88	1.98	1.91	2.61	2.76
0.70		1.78		0.90	0.87
0.67	0.73	1.72	1.82		
1.44		0.48		1.74	1.92
1.40	1.85	0.45	0.42	1.00	0.99
0.68		2.60		1.04	1.05
0.71	0.74	2.57	2.45	1.42	1.50
1.26	1.37	1.18		1.34	1.40
0.21	0.22	1.22	1.29		
		2.20		0.42	0.53
6.5	6.72	2.24	2.16	1.42	1.35
1.0	0.86				

\*According to Benedict's quantitative procedure.

# SERUM VEAL AGAR: A DEPENDABLE SUBSTITUTE FOR ASCITIC OR BLOOD AGAR\*

BY N. S. FERRY, M.D., AND ARLYLE NOBLE, A.B., DETROIT, MICH.

FOR reasons obvious to those interested in the routine culture of a large number of organisms, and especially mass growths, it was necessary to find, if possible, a substitute for ascitic or blood agar. Consequently a large number of different media were tested and it was determined that a veal agar (neutral to phenolphthalein) to which had been added normal horse serum (or any other serum) would successfully accomplish the purpose.

It was found, also, that the organisms would grow, in most instances, as well, if the peptone were omitted from the formula. This is an exceedingly practical point, especially where large quantities of peptone are consumed. (See "Peptone-free Media for Routine Culture Work," page 298 of this issue.)

## FORMULA FOR SERUM VEAL AGAR

1. To 500 grams of lean chopped veal add 1,000 c.c. water. Macerate and allow to stand in refrigerator 24 hours. Strain through cheesecloth and bring to boil.

2. Filter, add 20 grams peptone (this may be omitted), 5 grams NaCl, and 30 grams finely chopped agar-agar.

3. Boil and adjust reaction to the neutral point with phenolphthalein (the success of the results with the medium depends in a large measure upon this point).

4. Filter and pour into test tubes, 3 c.c. each.

5. Sterilize fractionally, cool to about 45° C. and add 2 c.c. of sterile normal horse serum to each tube. The preparation should be about three parts of agar to two parts of serum.

## ORGANISMS TESTED

1. *Diplococcus pneumoniae*, Types I, II and III, cultures of which had been kept on whole blood (rabbit) agar. The majority of these cultures had recently been isolated.

2. *Streptococcus viridans* and *hemolyticus*, cultures of which had been kept on whole blood agar.

3. Various strains of streptococci which had been cultured on ascitic agar. The majority of these were fresh cultures from tonsils.

The following charts will convey some idea of the results of the tests. The figures in the charts are to be interpreted as follows:

1 or 1+ = Normal growth and is to be considered first choice.

1- = Abundant growth, but not quite as good as on control media.

2 = Moderate growth.

3 = Poor or slight growth.

All readings were made after 24 hours at 37.5° C. The cultures were then

---

\*From the Research Laboratory, Parke, Davis & Co., Detroit, Mich.

	GROWTH ON														
	WHOLE BLOOD AGAR					ASCITIC AGAR					SERUM VEAL AGAR				
Number of generation after experiment starts.....	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
Number of days in ice chest between transplants.....	4	4	6	14	17	4	4	6	14	17	4	4	6	14	17
<b>Pneumococcus</b>															
No. Type															
3 I.....	1	1	1	1	1	3	2	3	—		2	2	1	1	2
4 III.....	1	1	1	1	1	3	2	1	—		1	1	1	1	1—
5 I.....	1	1	1	1	1	2	1	1	3	2	1	1	1	2	1
6 II.....	1	1	1	1	1	3	1	2	2	2	2	1	1	1	1
7 III.....	1	1	1	1	1	2	2	2	2	2	1	1	1	1	1
8 IV.....	1	1	1	1	1	3	1	2	1	1	1	1	1	1	1
9 III.....	1	1	1	1	1	3	1	1	—		1	1	1	1	1
10 I.....	1	1	2	—		3	3	3	—		3	2	1	2	2
11 II.....	1	1	1	3	2	3	3	2	—		2	1	1	2	2
12 III.....	1	1	1	1	1	2	1	2	1	3	1	1	1	1	1
13 I.....	1	1	1	2	1	3	3	3	—		2	1	1	1	2
15 II.....	1	1	1	1	1	2	2	1	3	2	1	1	1	1	1
16 I.....	1	1	1	3	3	3	2	2	—		2	1	1	1	2
17 I.....	1	1	1	—		3	3	3	—		3	2	1	2	2
18 I.....	1	1	3	—		3	3	2	—		3	2	1	2	2
19 I.....	1	1	3	—		3	3	3	—		3	2	1	2	2

	GROWTH ON									
	ASCITIC AGAR					SERUM VEAL AGAR				
Number of generation after experiment starts.....	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
Number of days in ice chest between transplants.....	6	6	14	14	14	21	6	6	14	21
<b>Streptococcus from tonsils</b>										
No. 1 (a).....	1	1	1	1	1	1	2	2	1	1
1 (b).....	1	1	1	1	1	1	2	1	1—	1
3 (a).....	1	1	1	1	1	1	1	1	1	1
3 (b).....	1	1	1	2	1	1	1	1	1	1
13.....	1	1	1	2	1	1	1	1	1	1+
14.....	1	1	1	1	1	1	2	1	1—	1+
15.....	1	1	1	1	1	1—	1	1	1+	1+
22.....	1	1	1	1	1	1	1	2	1	1
33.....	1	1	1	1	1	1	1	1	1	1
43.....	1	2	1	2	1	3	2	1	3	1
45.....	1	1	1	1	1	1	2	1	2	1
56 (a).....	1	1	1	1	1	1	2	1	2	1



placed in the refrigerator, as has been the routine custom for ascitic or blood agar cultures, for the time indicated on the charts, ranging from four to seventeen days.

	GROWTH ON														
	WHOLE BLOOD AGAR					ASCITIC AGAR					SERUM VEAL AGAR				
Number of generation after experiment starts.....	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
Number of days in ice chest between transplants.....	14	6	14	14	14 21	14	6	14	14	14 21	14	6	14	14	14 21
<i>Streptococcus viridans</i>															
No. 1.....	1	1	1	1	1	1	2	1	1	3	1	1	1	1	1
2.....	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1
3.....	1	1	1	1	1	1	3	3	1	1	1	1	1	1	—
4.....	1	1	1	1	2	3	1	3	—	—	3	1	1	1	1—
5.....	1	1	1	1	1	1	1	2	1	1—	1	1	1	1	1
6.....	1	1	1	1	1	2	2	2	2	3	2	1	1	1	3
7.....	1	1	1	1	1	1	2	2	1	3	1	1	1	1	1—
8.....	1	1	1	1	1—	1	3	1	—	—	1	1	1	1	—
9.....	1	1	2	1	3	2	3	2	—	—	1	1	2	1	1—
10.....	1	1	1	1	1	2	3	3	2	—	1	2	1	1	3
11.....	1	1	1	1	3	1	2	3	1	—	1	1	3	1	3
12.....	1	1	1	1	2	2	1	2	2	—	2	1	1	1	3
<i>Streptococcus hemolyticus</i>															
No. 1.....	1	1	1	1	1	1	1	1	1	1	2	1	1	1+	1
2.....	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3.....	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4.....	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5.....	1	1	1	1	1	1	1	1	1	1	2	2	2	1	1
6.....	1	1	1	1	1	1	1	1	1	1	2	2	2	1	2
7.....	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8.....	1	1	1	1	1	1	1	1	1	1	3	2	1	1	1
9.....	1	1	1	1	1	1	1	1	1	1	2	2	2	1—	1
10.....	1	1	1	1	1	1	1	2	1	1—	1	1	1	1	1
11.....	2	1	1	2	2	3	3	2	3	—	2	1	1	—	1
12.....	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

## CONCLUSIONS

1. Cultures of *diplococcus pneumoniae* grow as well on serum veal agar, after the second generation, as on whole blood agar and far better than on ascitic agar. These cultures lived longer on serum veal agar at refrigerator temperature, without transplanting, than on whole blood agar or ascitic agar. After two weeks four out of sixteen cultures on whole blood agar and ten out of sixteen on ascitic agar were dead, while all on serum veal agar were alive after seventeen days, and grew well when transplanted on the same media.

2. Cultures of streptococci grew as well and lived as long on serum veal agar as on whole blood agar and better than on ascitic agar.

3. Serum veal agar (veal agar, 2 per cent peptone, neutral, plus normal horse serum) can be used for maintaining cultures of most organisms ordinarily kept on ascitic or whole blood agar.

## PEPTONE-FREE MEDIA FOR ROUTINE CULTURE WORK\*

BY N. S. FERRY, M.D., AND ARLYLE NOBLE, A.B., DETROIT, MICH.

AS a result of some experiments which necessitated the use of culture media prepared without peptone, it was found that the organisms under observation grew as luxuriantly as on standard media, suggesting the possibility of employing such media for routine purposes. It was determined, therefore, to give the method a comprehensive trial, as it involved a question of practical nature, especially to laboratories where large quantities of media are consumed daily.

Ten different kinds of media were prepared without peptone, part of them neutralized and part made 1.0 per cent acid, phenolphthalein being used as the indicator.

1.	Liebig's Ext.	bouillon—without	peptone—neutral.
2.	"	"	" —1.0% acid.
3.	Beef	bouillon	" —neutral.
4.	"	"	" —1.0% acid.
5.	"	agar	" —neutral.
6.	"	"	" —1.0% acid.
7.	Veal	bouillon	" —neutral.
8.	"	"	" —1.0% acid.
9.	"	agar	" —neutral.
10.	"	"	" —1.0% acid.

A large variety of organisms was grown on the above media, culturing for several generations both freshly isolated strains and strains of various ages. For comparison, the same strains were grown on the standard media with Witte's peptone.

A tabulation of some of these results is given.

The figures in the charts are to be interpreted as follows:

1+ = Better than normal (on control media).

1 = Normal, abundant growth; first choice (except for an occasional 1+).

1- = Abundant growth, but not quite normal; second choice.

2+ = Abundant growth; third choice.

2 and 2- = Moderate growth.

3 = Poor growth.

4 = Very slight growth.

When two or more culture media are represented by the same figure, it indicates that there was no choice between them.

All results were read at the end of twenty-four hours. With each organism several generations were watched, not only in the incubator, but also at 5° C., so that the conclusions were not arrived at after a single generation at 37° C.

\*From the Research Department, Parke, Davis & Co., Detroit, Mich.

## GROWTH IN BOUILLON

ORGANISMS	MEDIA WITH WITTE'S PEPTONE			MEDIA WITHOUT PEPTONE														
	Plain Bouillon			Liebig's Ext. Bouillon		Veal Bouillon						Beef Bouillon						
	1.0% Acid			Neut- ral	1.0% Acid	Neutral			1.0% Acid			Neutral			1.0% Acid			
	1st gen.	2nd	4th	1st gen.	1st	1st gen.	2nd	4th	1st	2nd	4th	1st	2nd	4th	1st	2nd	4th	
Staphylococcus aureus	1	1	1	3	4	1	1	1	2+	1	1	1	1	1	1	2+	1	1
Staphylococcus citreus	1	1	1-	3	4	1	1	1	2+	1	1	1	1	1	1	2+	1	2+
Staphylococcus albus	1	2-	3	2+	-	1	2+	1-	1	1	1	1	2	2	1	1	2+	2+
B. pyocyaneus.....	1	1	1	2+	-	1	2+	1-	1	1	1	1	2+	1	1	1	2+	2+
B. coli.....	1	1	1	3+	-	1	1	1	1	1	1	1	1	1	1	1	1	1
B. paracoli.....	1	1	1	3	4	1	1	1-	1	2+	1	1	1	2+	1	2+	2+	2+
B. typhosus.....	1	1	1	3	4	1+	1+	1+	2+	2+	2+	1	1	1	1	2+	2+	2+
B. paratyphosus A...	1	1	1-	3	4	1	1+	1	2+	2+	2+	1	1	1	2+	2+	2+	2+
B. paratyphosus B...	1	1	1	3	4	1-	1	1	2+	1	2+	2+	1	1	2+	2+	2+	2+
Streptococcus.....	1-	1-	1	4	4	1+	1+	1+	2+	1	1	1	1	2+	1	2+	1	1

1st generation planted from 24-hour growths on agar into the above bouillons.

2nd transplanted from the 24-hour bouillon growths.

4th transplanted from third generation after being grown 24 hours and then placed in ice chest for 48 hours.

## GROWTH ON AGAR

ORGANISMS	MEDIA WITH WITTE'S PEPTONE			MEDIA WITHOUT PEPTONE											
	<i>Plain Agar</i>			<i>Veal Agar</i>						<i>Beef Agar</i>					
	1.0% Acid			Neutral			1.0% Acid			Neutral			1.0% Acid		
	1st gen.	2nd	4th	1st	2nd	4th	1st	2nd	4th	1st	2nd	4th	1st	2nd	4th
<i>Staphylococcus aureus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Staphylococcus citreus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Staphylococcus albus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>B. pyocyaneus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>B. coli</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>B. paracoli</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>B. typhosus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>B. paratyphosus A.</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>B. paratyphosus B.</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Streptococcus</i>	1	1	1	1	1	1	3	2+	1	2+	1	1	1	2+	1
<i>B. diphtheriae</i>	1	1	1	1	1	1	2+	2	2+	1	1	2+	1	1	2+

1st generation transplanted from 24-hour growths on plain, aseptic and Loeffler's Blood serum agar.

2nd generation transplanted from first after 24 hours.

4th generation transplanted from third after being grown 24 hours and then placed in ice chest for 6 days.

## CONCLUSIONS

1. On peptone-free agar all cultures which are ordinarily grown on standard plain agar did so well that it was difficult to choose.

2. For the majority of cultures, veal broth media gave better results than beef broth, both with and without agar.
3. Both veal and beef broth gave far better results than Liebig's beef extract.
4. Throughout the whole experiment the neutral media gave better results than the 1.0 per cent acid.
5. Therefore, for organisms which grow readily on standard plain agar, neutral veal or beef media, without peptone, can be substituted.

---

## A SIMPLE TEST FOR GLYCURONATES IN THE URINE

BY FRITZ C. ASKENSTEDT, M.D., LOUISVILLE, KY.

---

AS a result of the activities of the intestinal flora a number of toxic products of the aromatic series, such as indol, skatol, cresol, and phenol, are to be found in greater or lesser amounts in the human intestine. Much of these products is eliminated with the feces, while a part becomes absorbed into the portal circulation. The toxic action of these aromatic bodies is neutralized by conjugation with sulphuric acid or with glycuronic acid in the liver. Thus are formed, on the one hand, indoxyl-glycuronic acid, skatoxyl-glycuronic acid, cresol-glycuronic acid, phenol-glycuronic acid; and, on the other, corresponding combinations with sulphuric acid, the so-called ethereal sulphates. That a part of these absorbed basic substances is destroyed by oxidization seems probable, another part undergoes different chemical transformations (hippuric acid, indol-acetic acid, etc.), but the largest portion is excreted by the kidneys as above ethereal sulphates and glycuronates.

Except in cases of nephritis, indicanuria affords a fair index to the absorption of indol, which is formed from tryptophan mainly in the lower small intestine, but it bears no direct relation to the absorption of phenol, whose formation from tyrosin in the colon is usually slightly in excess of that of indol. While, as a rule, an excessive indol formation is attended by an excessive production of all the other above mentioned putrefactive products, it will readily be seen that owing to a difference in the character of the protein ingested, and possibly to a difference in the location of an intestinal lesion impeding peristaltic propulsion, the quantitative relation between the indol and the phenol productions may present considerable variations. As evidence of intestinal putrefaction—barring cases of putrefactive abscesses—a urinary test that will indicate the absorbed quantity of these two or more benzene derivatives is, therefore, ordinarily of greater utility than the test which represents but one factor. For such a purpose Folin's test for ethereal sulphates stands unchallenged in scientific value, but its execution demands considerable time, technic, and equipment, which necessarily confines its use to the larger laboratories. The glycuronates, which are capable of intense color reactions, are better suited for simple clinical tests. After some years of experimentation with Goldschmiedt's and Clifford Mitchell's reagent, alpha-naphthol (Modern Urinology), I feel warranted



in presenting the following test for glycuronates, which combines simplicity with a fair degree of accuracy:

The urine to be examined is diluted with water until its specific gravity is reduced to 1.001. For example, if the urine has a sp. gr. of 1.015 (correction being made for temperature), dilute 1 c.c. of urine with 14 c.c. of water; if the sp. gr. is 1.021, dilute 1 c.c. with 20 c.c. of water. (Diabetic urine should be diluted until its urea content is 0.1 per cent.) To 10 c.c. of the diluted urine in a test tube add one or two drops of a 1 per cent solution of alpha-naphthol in glycerin (alcohol impairs the test), and then 10 c.c. hydrochloric acid, sp. gr. 1.19. Mix by turning the tube over once or twice. Then let the tube stand in a dark, cool place for about twelve hours, after which the reaction is noted in reflected light. This is best done by holding a white surface, as, for example, a white blotter, behind the tube. Normally the fluid will remain colorless or show a mere suggestion of blue. If glycuronates are present in excess, there will appear a proportionate blue color, tinged with red. The depth of the color may be denoted by the customary use of plus signs, medium blue, the deepest color observed in the above dilution, being designated with ++++. If greater accuracy of estimation is desired, a standard solution of indigo blue, containing some indigo red, in sulphuric acid, may be employed by dropping it into another test tube of the same size, containing 20 c.c. of water. The tube receiving the standard solution is turned over after each drop is added and comparison of the two tubes is made, this procedure being continued until the nearest likeness of color is obtained, when the number of drops added is recorded. In twenty-four hours, or later, a slight cloudiness, with fading of color, appears, ultimately forming a sediment.

This test proves less sensitive to contaminating gases in the laboratory than do the indigo tests for indican. The slow reaction is its greatest disadvantage. Heating the mixture destroys or seriously impairs the reaction. Diabetic urine responds normally to the test, except that the blue color assumes a somewhat muddy hue. When first stale the urine reacts more promptly to the test than when fresh, but since by the action of putrefactive bacteria glycuronic acid is decomposed, and since certain compounds, as menthol- or thymol-glycuronic acid have been found to undergo spontaneous disintegration, the urine should preferably be fresh when tested.

Pentose in the urine may possibly give a bluish reaction. As I have been unable to procure any form of pentose in the market for experimental purposes, I can not at present produce any evidence bearing on this question. However, since pentosuria is a rare condition, and acute cases can usually be avoided by having the patient abstain for a few days from fruit, fruit juices, and malt liquors in the diet, it can not seriously detract from the value of the test. Moreover, a difference in the behavior of glycuronates and that of pentoses in the orcin test\* will reveal the presence of pentosuria when suspected.

The presence of nitrites in the urine will inhibit the reaction of glycuronates,

\*The urine is mixed with an equal volume of concentrated hydrochloric acid, a few small crystals of orcin are added and the mixture is then heated. As soon as the fluid becomes green or bluish green, it is cooled until lukewarm and shaken with amyl alcohol, which will render it bluish green (positive reaction).

Solutions of glycuronates, unlike pentoses, do not react to the orcin test directly, but only after the glycuronates have been disintegrated by boiling with an acid and their reaction is, therefore, several minutes tardier. (Olof Hammarsten, *Fysiologisk Kemi*, ed. 2, 358, 359.)

but may be suspected by their giving to the test fluid, immediately upon adding the hydrochloric acid, a light yellow tint.

It must be kept well in mind that the production and excretion of glycuronates are greatly augmented by ingestion of the following drugs, the toxicity of which becomes neutralized by paring with glycuronic acid: chloral hydrate, camphor, turpentine, morphine, menthol, arsenic, acetanilide, antipyrin, benzol, chloroform, curare, hydroquinone, naphthalin, phenol, resorcin, salicylic acid, salol, sulphonol, trional, and thymol. These remedies should, therefore, not be employed before the urine is collected for examination. It seems probable that a comparison of the reaction for glycuronates with the result of a reliable indican test will afford a valuable indication in cases of suspected poisoning with any of the above drugs, but opportunities for a clinical demonstration of this suggestion have not been afforded me.

The statement made by Schmiedeberg that glycuronates are formed only when sulphur is no longer available to the liver for the production of ethereal sulphates, seems incorrect, for glycuronates can always be demonstrated in undiluted urine, even when indican is absent.

In estimating the clinical value of the test it must be remembered that the excretion of an unusual amount of glycuronates or indican may be consistent with health. So long as the liver is functionally adequate to neutralize the entire amount of the toxic materials brought to it by the portal vein, little or no harm results, but a constant overstimulation of any function of the body tends to ultimate insufficiency, and a constant excessive production of indican and glycuronates is a positive signal of present or approaching danger.

---

## AN ANTIGEN FOR USE IN COMPLEMENT FIXATION IN TUBERCULOSIS\*

BY MOYER S. FLEISHER, M.D., AND GEORGE IVES, M.D., ST. LOUIS, MO.

---

IT is not our intention to report here on the clinical value of this antigen or to discuss the significance of the complement-fixation reaction in tuberculosis. We desire only to present this antigen and state some reasons for our belief that it has a definite value in the recognition of tuberculous infection. The method of preparing the antigen is as follows:

Tubercle bacilli are isolated and grown from sputum on Petroff's medium. When the cultures show an abundant growth, forming definite raised masses, the organisms are scraped from the surface of the medium with a heavy platinum wire. It takes from four to eight weeks for the growth to become sufficiently abundant. The tubes of media are plugged in the ordinary manner. Every few days a portion of a cubic centimeter of water is added to the tubes. In all cases a number of different strains of organisms are used in making one antigen, and the number has varied from four to ten strains.

---

\*From the Department of Pathology and Bacteriology, St. Louis University School of Medicine, and the Clinical Laboratory of George Ives.

The organisms are placed in a sterile open Petri dish or watch glass and allowed to dry overnight in a bacteriologic incubator at 37° C. It is not necessary to keep the mass of tubercle bacilli free of contamination, but contamination should be avoided as much as possible. The partially dried bacteria are weighed and then placed in a large, sterile, clean mortar. They are ground for three to four hours, vigorously and constantly. From time to time during the grinding, a scalpel is used to scrape the bacterial mass from the sides of the mortar and from the pestle. A few drops or as much as 1 c.c. of sterile distilled water is added when necessary to keep the mass of the proper consistency. Addition of sufficient water to make a fluid mass should be strictly avoided.

After grinding the necessary length of time, 0.85 per cent sodium chloride solution is added little by little, and the grinding is continued until the bacteria or remains of the bacteria form an even suspension in the diluent. Sufficient of the sodium chloride solution is added to make a 0.5 per cent suspension of bacteria. To this there is added an amount of 5 per cent carbolic acid equal to one-tenth the volume of the sodium chloride solution.

The finished antigen is dense, grayish, and opaque. The bacterial bodies and suspended cell-remains settle to the bottom, if the suspension is allowed to stand. The supernatant fluid is very slightly opalescent. Before use the antigen should be shaken so that the solid parts are again brought into suspension.

In the making of the antigen the most important step is the grinding. It has been demonstrated that less than several hours of proper grinding gives an antigen which is worthless or at least too weak for use in the method of complement fixation which we have used. Possibly if the grinding is continued for a longer time than we recommend, a more efficient antigen may be obtained.

The considerations which underlie the first attempts to make this antigen are largely theoretic. It seemed from a review of the literature that of all antigens used previous to the middle of 1915, the bacillary emulsions or antigens similar to these had given the more favorable results, with the possible exception of Besredka's antigen. It further appeared that there exist in the tubercle bacillus several partial antigens;\* that different constituents of the tubercle bacillus call forth different antibodies which appear in the blood of the tuberculous individual at varying periods and in varying quantities. In preparing an antigen for the complement-fixation test, one should therefore attempt to obtain in that antigen all the various antigenic constituents of the tubercle bacilli. Neither an extract nor a filtrate do in all probability contain all these antigens. Although living and morphologically unmodified tubercle bacilli do contain them, in complement-fixation reactions they are possibly prevented from union with their corresponding amboceptors of the patient's serum by the waxy capsule of the bacillus. By grinding we may liberate water soluble antigenic constituents. If the theoretical considerations upon which this antigen was first prepared are supported by experiment, it must be concluded that this antigen is not included in the classification of antigens given by Miller.<sup>1</sup> We hoped to prepare, and evidence given below seems to indicate that we did prepare, an antigen which is both a suspension and a watery extract of tubercle bacilli.

We know that parasitic organisms may lose their virulence, and change in

\*For the present discussion, we accept the correctness of the views of Much and Deycke.

their biological characteristics in other ways when they are cultivated on artificial media for several generations. This change in function is probably indicative of a change in metabolism, and probably of chemical composition. If, therefore, one desires to use as an antigen an organism as nearly as possible identical with the bacteria acting in the human body, it is essential to use an organism freshly isolated from the human. The use of the freshly isolated tubercle bacillus grown on Petroff's medium represents the simplest means of realizing the desired end.

It appeared furthermore that in order to obtain an antigen containing substances differing as little as possible from those of the actively pathogenic organisms, it would be necessary to avoid subjecting the bacilli to any agent which might lessen or destroy their antigenic qualities. We know that coagulation such as may be effected by heat, and coagulation such as may be brought about by some germicides, changes, at least in a small degree, the reactive properties of most antigens. Because of this consideration, the organisms were not killed nor modified in any manner before they were used for grinding. The use of several strains of tubercle bacilli in making the antigen is, of course, logical from the point of view of the individuality of various strains of organisms.

We are quite willing to admit the possibility that these theoretic considerations, which determined the method of making this antigen, may not have the significance that we attach to them. Whether the use of freshly isolated organisms, the avoidance of changing the bacilli in a manner or degree other than that involved in our method, the use of several strains of organisms, or the use of the entire organism, are essential considerations in the production of the most suitable antigen, must be determined by further experiment.

The power of our antigen to fix complement in the presence of the sera of tuberculous individuals resides, not only in the entire antigen, but in both the clear supernatant fluid and in the suspended matter. In an experiment with a series of cases in which parallel tests were made using as antigens the supernatant fluid as one antigen and the entire antigen as the other, corresponding results were obtained. The supernatant fluid is a suitable antigen, but it is weaker than the entire antigen. This experiment in itself would seem to distinguish this antigen from the Miller<sup>1</sup> antigen which according to Corper<sup>2</sup> shows little or no antigenic properties in its fluid portion. While the first antigen made by this method was produced in February, 1916, and while it was tested out in a few cases at that time, it was not until late in that year that extensive work was taken up with this antigen by one of us (George Ives) and sufficient cases were collected to warrant a definite idea of its value.

We believe that one can obtain with this antigen results equal to those obtained with any of the antigens which have been proposed by others. A series of cases have already been reported,<sup>3</sup> and since the publication of this report, a large number of tests have been performed which yielded results closely paralleling those of the reported series.

Comparing our results and interpretation of the test, we find ourselves in close harmony with Bronfenbrenner<sup>4</sup> and McCaskey.<sup>5</sup> The close parallelism between the results of the complement-fixation test for tuberculosis and clinical findings which have been reported by Miller<sup>1</sup> and Craig<sup>6</sup> we believe may well



not exist when a delicate test is applied for the recognition of tuberculosis. The very nature of tuberculosis teaches us that such a parallelism can not exist when the test is applied to a large group of individuals. The most striking instance of this lack of parallelism between clinical and serologic findings occurs in syphilis. In our unpublished data we find that over 50 per cent of syphilitics with a positive Wassermann give a positive complement-fixation test for tuberculosis. Most of these are cases of latent syphilis, and very few of them present any symptoms suggestive of tuberculosis.

We believe that this antigen possesses two further merits which have not been claimed for other antigens. First, owing to the addition of carbolic acid, it is sterile and remains sterile; second, it is stable, changing little if at all in its antigenic titer over a long period of time. At least one sample of antigen gave excellent results when used over a year after its preparation, and none of several antigens have shown deterioration after considerable lapses of time.

There is one other consideration which may give further evidence of the value of this antigen. For the past several months in the performance of the complement-fixation test, we have altered our technic and now titrate both complement and amboceptor each time the test is performed. We use a method of titration based upon a suggestion of Williamson.<sup>7</sup> We use in the test 1.5 units of complement and two units of amboceptor. We use heated patient's serum. Possibly our technic is not so delicate as that of the majority of workers in this field. If this is true, and if it be proved that our results with the complement-fixation test are as reliable as the results of those who use a more delicate technic, then it would appear that we have used a more sensitive antigen.

#### BIBLIOGRAPHY

<sup>1</sup>Miller: Jour. Lab. and Clin. Med., 1916, i, 816.

<sup>2</sup>Corper: Jour. Am. Med. Assn., 1917, lxviii, 1598.

<sup>3</sup>Ives and Singer: Jour. Missouri Med. Assn., 1917, xiv, 284.

<sup>4</sup>Bronfenbrenner: Jour. Lab. and Clin. Med., 1917, iii, 50.

<sup>5</sup>McCaskey: Am. Jour. Med. Sc., 1917, cliv, 648.

<sup>6</sup>Craig: Am. Jour. Med. Sc., 1915, cl, 782.

<sup>7</sup>Williamson: Jour. Lab. and Clin. Med., 1917, ii, 658.

# *The Journal of Laboratory and Clinical Medicine*

VOL. III.

FEBRUARY, 1918

No. 5

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.  
Ann Arbor, Mich.

## ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D.	- - -	ST. LOUIS
HANS ZINSSER, M.D.	- - -	NEW YORK
PAUL G. WOOLLEY, M.D.	- - -	CINCINNATI
FREDERICK P. GAY, M.D.	- - -	BERKELEY, CAL.
J. J. R. MACLEOD, M.B.	- - -	CLEVELAND
ROY G. PEARCE, M.D.	- - -	CLEVELAND
ROGER S. MORRIS, M.D.	- - -	CINCINNATI
GERALD B. WEBB, M.D.	- - -	COLORADO SPRINGS
E. E. SOUTHARD, M.D.	- - -	BOSTON

Contents of this Journal Copyright, 1918, by The C. V. Mosby Company—All Rights Reserved  
Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

## EDITORIALS

### *Goldberger's Studies of Pellagra*

FOR some years the United States Public Health Service has carried on an investigation into the etiology and epidemiology of pellagra and this work has been done largely, at least recently, under the direction of Goldberger. When this investigator began his inquiries, he was impressed with the following facts so forcibly that they determined the direction of his investigations.

It is the unanimous testimony of all who have observed and written about this disease that there is no evidence that it is ever transmitted to nurses, physicians, or attendants. In Italy where the disease has long been known and where special institutions have been provided for pellagrins, such transmission is unknown or at least unrecorded. No precautions have been taken to avoid transmission and still it has not occurred. This is confirmed by those who have made observations in this country. This seems to be admitted by all, even those who still claim that it is of bacterial or protozoal origin. Siler and Nichols, who studied the outbreak in the Peoria State Hospital some years ago, stated that no nurse, attendant, or employee of the institution at any time showed any evidence of the disease. In the Georgia State Sanitarium, where the sanitary

conditions have never been the best and where many of the wards are infested with cockroaches and bedbugs, and where this disease frequently develops among the patients, there has never been a case among physicians, nurses, attendants, or employees. This holds good for other southern insane asylums, such as the South Carolina and Mississippi State Institutions.

Impressed by these facts, Goldberger wrote in 1914 as follows: "In considering the significance of the foregoing observations, it is to be recalled that at all of these institutions the ward personnel, nurses and attendants spend a considerable portion of the twenty-four hours on day or night duty in close association with the inmates; indeed, at many of these institutions for lack of separate building or special residence for the nurses, these live right in the ward with and of necessity under exactly the same conditions as the inmates. It is striking, therefore, that although many inmates develop pellagra after varying periods of institutional residence—some even after ten to twenty years—which would lead one to believe it to be due to exciting causes within the institution, yet nurses and attendants living under identical conditions appear uniformly to be immune. If pellagra be a communicable disease, why should there be this exemption of nurses and attendants? To the writer this peculiar exemption or immunity is inexplicable on the assumption that pellagra is communicable. Neither contact in any sense nor insect transmission is capable of explaining such a phenomenon, except on the assumption of an incubation or latent period extending over ten to twenty years. In support of such an assumption, there exists, so far as the writer knows, no satisfactory evidence. The explanation of the peculiar exemption under discussion may be found in the opinion of the writer in a difference in the diet of the two groups of residents. At some of the institutions there is a manifest difference in this regard; in others, none is apparent. The latter would seem to be a fatal objection to this explanation, but a moment's consideration will show that such is not necessarily the case. The writer from personal observation has found that although the nurses and attendants may apparently receive the same food, there is nevertheless a difference in that the nurses have the privilege, which they exercise, of selecting the best and the greatest variety for themselves. Moreover, it must not be overlooked that nurses and attendants have opportunities for supplementing their institutional dietary that the inmates as a rule have not."

Pellagra is a rural disease and one usually of poverty. In other words, pellagra is frequent among the rural poor and rare among the urban poor. This is a striking characteristic and evidently it has some meaning. There is great poverty among certain classes in all large cities and still pellagra is distinctly rural. Goldberger points out that the chief difference in the diets of the rural and urban poor lies largely in the greater sameness in the former and the greater variety in the latter. The urban poor may buy any day a penny's worth of fresh meat or milk while the rural poor can not buy these articles because they are not for sale in their community. It is therefore reasonable to conclude that variety in diet is desirable in order to prevent the development of pellagra. In other words, the disease is not due to a diet deficient in calories, but to one deficient in certain food principles.

On this point Goldberger writes: "With regard to the question of just

what in the dietary is responsible, the writer has no opinion to express. From a study of certain institutional dietaries, however, he has gained the impression that vegetables and cereals form a much greater proportion in them than they do in the dietaries of well-to-do people, that is, people who are not as a class subject to pellagra. The writer is satisfied that the consumption of corn or corn products is not essential to the production of pellagra, but this does not mean that corn, the best of corn, or corn products, however nutritious and however high in caloric value they may be, are not objectionable when forming of themselves or in combination with other cereals and with vegetables a large part of the diet of the individual. In view of the great uncertainty that exists as to the true cause of pellagra, it may not be amiss to suggest that pending the final solution of this problem it may be well to attempt to prevent the disease by improving the dietary of those among whom it seems most prevalent. In this direction, I would urge the reduction in cereals, vegetables, and canned foods that enter to so large an extent into the dietary of many of the people in the South, and an increase in the fresh animal food component, such as fresh meats, eggs, and milk."

At this point in his inquiry, Goldberger stated his conclusions as follows:

- (a) Pellagra is not a communicable disease, but is of dietary origin.
- (b) It is dependent upon some as yet undetermined fault in the diet in which the animal or leguminous protein component is disproportionately small and the nonleguminous vegetable component disproportionately large.
- (c) Pellagra does not develop in those who consume a mixed, well-balanced, and varied diet, such, for example, as that furnished by the Government to the enlisted men of the Army and Navy.

In 1915, twelve convicts in the Mississippi State Prison were offered pardons by the Governor on condition that they submit to an attempt to produce pellagra in them by dietary means. One of these developed a prostatitis and was eliminated from the squad. The eleven were kept on a pellagrous diet from February 4 to October 31, 1915. Six developed between September sixth and twenty-fourth, typical dermatitis, justifying a diagnosis of pellagra. The nervous and gastrointestinal symptoms were mild but distinct. The diet consisted wholly of vegetable foods poor in protein and with the exclusion of milk, legumes and fresh meat. The convicts who were on the ordinary prison diet showed no sign of the disease at any time, before, during, or after this experiment. This experiment seems to have demonstrated quite conclusively that pellagra may be induced in healthy men by diet. This test would have carried more weight could it have been made in a region where pellagra is not so prevalent, but since there had never been a case on the prison farm, we are inclined to accept it at full value. In our opinion, its importance is greatly increased by the observations to be detailed later.

Goldberger and Waring describe an observation made by them at two orphanages at Jackson, Mississippi, as follows: "The first of these two to be considered is spoken of as Orphanage M.J. Cases of pellagra had been recognized at this institution every spring for several years. During the spring and summer of 1914, up to September 15, 79 cases of the disease were observed in



children at this orphanage. Although several of these were known to have had pellagra on admission or had developed it a short time after admission, a number appeared to have developed the disease for the first time after considerable periods of residence at this institution. The factor or factors causing pellagra and favoring its recurrence seemed, therefore, to be operative at this institution.

"The second of the orphanages, which will be referred to as Orphanage B.J., is located about half a mile east of Orphanage M.J. Here, as at M.J., cases of pellagra had been recognized every spring for several years. The writers are informed by the superintendent that a condition which he can not distinguish from that now called pellagra has occurred every year among the children ever since his connection with the institution, a matter of some twelve or thirteen years. From this description it is believed that there can be but little doubt that pellagra has prevailed in this institution almost if not quite, since its foundation in 1897.

"During the spring and summer of 1914, up to September 15, there were observed among the children in this institution 130 cases of pellagra. As at M.J. some of these were in recent admission; a large proportion, however, occurred in long-time residents.

"There appears to be little, if any, reason to doubt that the factor or factors causing the disease and favoring its recurrence have been operative at this institution for many years.

"At both institutions the hygienic and sanitary conditions found left much to be desired. Both were much overcrowded. The drinking water supply at each is drawn from the public supply. One is equipped with a water carriage sewerage system connected with that of the city; the other has the unscreened surface privy type of sewage disposal, and incidentally, we found here a great deal of soil pollution. At the very outset, it was requested that no change be made in hygienic and sanitary conditions and it is believed that these have remained as they were found and as they have been for several years.

"Since about the middle of September 1914, the diet at both orphanages has in certain respects been supplemented by the Public Health Service. At both institutions a very decided increase was made in the proportion of the fresh animal and of the leguminous protein foods.

"The milk supply was greatly increased. Provision was made to give every child under twelve years a cup of about seven ounces of milk at least twice a day. Those under six had it three times a day. Until the spring of 1915, the milk used was all fresh, sweet milk. In April of this year buttermilk was added to the diet. This was served at first only on alternating days to those over twelve years of age; later, when a sufficient supply became available, it was served at the midday meal to all.

"Eggs, except in cooking or for the sick, had previously not entered into the diet of the children. The writers prescribed at least one egg at the morning meal for each child under twelve years of age. It had been the custom to serve the children with fresh meat but once a week. Under the writer's direction, it was increased to three or four times a week.

"Beans and peas, which had been conspicuous in the diet only during the

summer and fall, were made an important part of nearly every midday meal at all seasons.

"The carbohydrate component of the institution diet was also modified. The breakfast cereal was changed from grits to oatmeal, partly because it was believed to be an advantage to reduce the corn element and in part because it was believed that the oatmeal would favor the consumption of milk. The corn element, though much reduced, was not wholly excluded. Cornbread was allowed all children once a week and grits to those over twelve years of age once or twice a week. Cane syrup or molasses, which it had been customary to serve freely at two or three meals each day, was for some weeks entirely excluded, and later allowed in small amounts at only three or four evening meals a week. The object in this was to reduce the proportion of the carbohydrate element."

The results of these alterations in diet may be summed up as follows: At M.J., 67 cases of pellagra completed the first anniversary of their attacks with no recognizable recurrence, nor were there any evidences of the development of the disease among the 99 nonpellagrous inmates.

In B.J., of 130 cases, 105 completed the anniversary with recurrence recognizable in only one. Of 69 nonpellagrins, not one developed the disease. In other words, aside from admissions during the year, only one case of pellagra existed in the two institutions, while of the 172 who lived on the improved diet for one year, there was only one recurrence at the end of that time. In our opinion it is unfortunate that at the end of the year and with the close of the supervision of the food, observation and record were wholly discontinued.

The observation conducted at the Georgia Sanitarium is more valuable because longer continued. This is a large insane asylum for both whites and blacks. Two wards, one for each race and both female, have been under the modified diet from December 1914, up to the present time (November 1917). No case of this disease has developed among nonpellagrins in these wards and there has been no recurrence of the disease among the pellagrins. In other words, the disease has been eliminated from these wards, while new cases and recurrences have appeared in other wards. There seems no reason why pellagra should not be eliminated from the whole institution under improved diet. It should be understood, however, that simply supplying proper food is not enough. It must be seen that every inmate gets it and eats it. It has been necessary to feed some of the insane with a tube.

The time after the provision of better food before improvement is noticeable is widely variable. The first evidence of betterment may show in the stomatitis, dermatitis or enteritis, generally in the improved condition of the mouth. Marked diarrhea is not a contraindication to a more liberal diet. Marked improvement in the skin lesions is not usually in evidence until the lapse of at least three months but finally these lesions quite disappear.

If diet be an important factor in the etiology of pellagra and since this disease is much more prevalent in the South than in the North, the comparative diets among the working classes in the two sections should be studied. Sydenstricker of the Public Health Service is engaged in this study and published a preliminary paper in 1915. This paper is based on data supplied by an investigation made by the British Board of Trade into the cost of living in American

towns in 1909, and a similar study made by the Federal Bureau of Labor in 1901. Sydenstricker says that the former developed the fact that the food price level in southern towns, weighted according to actual consumption in wage earners' families, was above the average in the rest of the country, the southern towns being from two to nine per cent higher than New York City. "Prices of the cheaper cuts of beef and of milk were in nearly every instance higher in southern towns than in the North. The conclusions indicated by the available evidence and in accordance with the principles of family income and expenditure already referred to, are that the wage earner's family in the South is at a greater economic disadvantage than in the northern states and that there is a greater pressure exerted in favor of sacrifices in diet in order to maintain an otherwise comfortable standard of living." Not only is animal food higher in price in the South but such foods are less widely distributed and consequently less available to the working classes. "The Bureau of Labor's data for 1901 showed that southern wage earners' families consumed over 25 per cent less protein foods, over 50 per cent more fats, and nearly 10 per cent more carbohydrates than northern families."

"For the meats and other protein groups of foods the geographic differences in consumption are significant. In the northern states the average family was found to consume annually between 1,000 and 1,100 pounds of protein, while in the southern states the protein consumption averaged between 700 and 800 pounds. The southern family consumed nearly a pound a week less of fresh beef, nearly half as much milk, very much less of other meats, and hardly any salt beef as compared with northern families. For the fats and hydrocarbon group of foods, even more significant differences are shown. While families in northern states were found to consume larger quantities of butter, families in southern states consumed over 60 per cent more lard and nearly three times as much salt hog products."

Sydenstricker has more recently collected a large amount of data bearing on the dietaries of the cotton mill employees, in relation to the incidence of pellagra in the different mill villages and in pellagrous and nonpellagrous families. When these facts are tabulated, they should prove of great value.

—V. C. V.

---

### *The Control of Communicable Disease Among Our Soldiers*

AT present, this is one of the many big problems that confront the nation and demand solution. So far as the Medical Department of the Army is concerned, it is the biggest problem. While the mortality in our camps during the past few months has not risen to the great height that it did in 1898, it is sufficient to cause anxiety and to demand that every effort possible be made to reduce it.

That failure to supply proper and adequate clothing early has been a most potent factor in the incidence of pneumonia among our soldiers certainly has been abundantly demonstrated. A man can be chilled occasionally and for short period of time without injury to his health, but when one is cold night and

day, even the most robust is liable to succumb to any infection that may present itself. The spread of infection in our tents and barracks has been favored by overcrowding. These untoward conditions are being removed. The Secretary of War finds that most of the soldiers have received in January the clothing which should have been provided at the latest early in October. The Surgeon-General asked in July for at least fifty square feet of floor space per man in tent and barrack. This request has at last been granted, and provision is being made for its supply. Detention camps for incoming soldiers, where they may be kept under observation before mingling with others, have been ordered. Furthermore, additional space and housing are to be available for the prolonged care of convalescents before their return to duty, for contacts, suspects, and carriers. These are wise provisions and will do much to reduce the prevalence of communicable diseases among our soldiers.

However, it will be necessary to go further than this before we reach the greatest possible accomplishment, and we should not be satisfied with less. In the transformation of civilians into soldiers, some lives must be lost, but it is certainly the intention and desire of the whole nation that this loss be reduced to a minimum. Up to the present time, infections have been most prevalent in southern camps, but the season for respiratory diseases in the northern states has not yet been reached. This generally falls in February and March, and, while no one wishes to prophesy evil, it is wholly within the range of probability that during these months the respiratory diseases will be markedly augmented in all the camps and cantonments in the United States.

It must be evident to all thinking men that the conservation of the health of the nation is of prime importance at all times, but especially so under the stress of war. Each individual incapacitated by disease among the civilian or the military population reduces national efficiency, not only so far as the efforts of this individual are concerned, but to the extent that the services of others are absorbed in his care. A sick soldier is, so long as he is ill, not only one less in the effective force, he can not drill or otherwise prepare himself for the fighting line, but he is a possible source from which infection may be spread to others, and his care occupies the attention and time of others. A sick civilian is one withdrawn for the time being from the great industrial army which must stand behind the fighting force, and his care also withdraws one or more from the same service. In short, and it needs no argument, this is a time when all preventable sickness should be prevented.

It must be recognized that in the preservation of the health of the nation, no sharp line can be drawn between civilians and soldiers. Civilians constitute the source of supply of soldiers, and soldiers are constantly mingling with and returning to the civilian population. The relation between these two bodies is one of fluidity and not solidity. Failure to see this and act accordingly may lead to disaster in both portions of our population. At present, civilians are constantly invading the camps in which the infection becomes intensified and is returned to the civilian population throughout the length and breadth of the country. The assembly of troops, whether it be by draft or by voluntary service, acts like a dragnet, bringing the widely scattered infections from city, village and farm, from every part of the compass and from every class and con-



dition of citizenship. The camp acts as an incubator in which the infections develop and through which they spread and increase in virulence. Furloughed and discharged soldiers distribute the infection incubated and intensified in the camps, and even those who are sent across the seas bear these infections to our troops in France. If we are to win this war with the least possible expenditure of life, we must control, or at least abate, the infection among our soldiers, and this can not be done without attention to the abatement of communicable diseases among the people as a whole.

Suppose we have an army of three million men. At the same time, we have a civilian population of ninety-seven million. These are indeed one population, mingling and mixing at all times; one being converted into the other and then returning to the original source. Common sense shows that it is not possible to stamp out disease among 3 per cent of our population while we neglect the source among 97 per cent.

The health organization of the country, both national and state, extending down to the locality, should be and must be, if our troops are to be most effective, under one and the same control. With this in view, the following recommendations are offered:

(1) The United States Public Health Service, with its personnel, functions, duties, properties including its hospitals, quarantine stations, etc., should be transferred to the Medical Department of the army, and placed within the jurisdiction and under the command of the Surgeon-General of the army during the continuance of the war.

(2) If the above recommendation can be put into effect, the Surgeon-General of the Army should assign to duty in each state a medical officer whose function should be to cooperate with the state health authorities in the control and abatement of communicable diseases among the entire population of the state, both civilian and military, both temporary and permanent residents.

(3) In case the first recommendation be put into effect, the Surgeon-General of the Army should station at such points in each state as he may think wise, and we suggest in the proportion of one to fifty thousand population, additional military health officers, who, under the direction of the state officer mentioned in number 2 shall cooperate with the local health authorities in their efforts to control and abate communicable diseases.

Fortunately, the first recommendation is already legally provided for. Section IV of an Act of 1902 reads as follows:

"That the President is authorized in his discretion to utilize the Public Health and Marine Hospital Service in times of threatened or actual war to such extent and in such manner as shall in his judgment promote the public interest, without, however, in any way impairing the efficiency of the service for the purpose for which the same was created and is maintained."

Under date of April 6, 1917, the President promulgated an order in accordance with the above mentioned provision in the law. It is only necessary for the Secretary of War to ask the Secretary of the Treasury to transfer, for the period of the war, the United States Public Health Service with all its functions, duties and properties, to the War Department.

The practical working of these regulations should be something like the following:

The local military health official should keep himself well posted on the communicable diseases in his district, and he should be vested with all authority necessary in the control of the same. He should have under his direct supervision and command, all new troops, both those volunteering and those drafted within his district. He should see that these men are not unnecessarily exposed to infection, and he should know to what infections and to what extent they have been exposed. From the time a young man is selected for the draft until he reaches the camp to which he is to be assigned, he should be under the direct charge, supervision and control of the local military health officer. When the squad from that vicinity is assembled, this official should either in person or by a deputy, conduct these soldiers to the camp to which they were assigned and turn them over to the medical officer of the camp with a full statement of all exposures to infection. When a soldier is furloughed from camp, he should be transferred to the command of the local health officer and should be under his charge, care, and control during the whole of the time of his furlough. The local military health officers should make immediate telephonic or telegraphic reports to the state officer, and in case the military camp or cantonment is located in that state, directly to the division surgeon.

—V. C. V.

---

### *Military Antituberculosis Program Perfected*

PLANS for a complete program for the prevention of tuberculosis in the army have been perfected by the National Association for the Study and Prevention of Tuberculosis working in cooperation with the Surgeon-General, the Y. M. C. A., and other agencies. This, it is predicted, will put the impending second draft on a better health basis than the first. The program will include not only a follow-up for every man discharged on account of tuberculosis, but a thorough-going health educational campaign among the soldiers.

Prior to the first draft the National Association began to outline a preventive campaign. Owing to the magnitude of the task and the many practical delays in perfecting and applying the details of this scheme, the results were not as encouraging as might be expected. This was due to the fact that the report of names of men rejected by the draft on account of tuberculosis was inadequate, the slowness of the machinery in getting under way, and the many difficulties in determining the status of the men.

Inasmuch as these enlisted or drafted men do not become accepted soldiers until after their probationary period lasting from three to six months in the various services, the government assumes no responsibility for the after-care of those whose health breaks down during that period. Hence, this problem belongs to the civilian boards of health and the unofficial health organizations.

The National Association program falls into two main divisions: (a) follow-up work and (b) educational work. The first obstacle to the follow-up program was Section Eleven of the Selective Service Regulations regarding the

second draft which forbids giving a record of a man's condition to anyone except certain designated officials. The National Association officers, however, placed before the War Department the importance of this work and were influential in persuading them to open the records of rejected men to state and local boards of health throughout the country, through the United States Public Health Service and the Council of National Defense.

Inasmuch as the above section of the regulations does not apply to men dismissed from training camps after they have passed draft boards, the association arranged with the Surgeon-General and the division surgeons in camps to receive the names of all men thus dismissed. These lists are divided up by states and forwarded to state associations and state boards of health for follow-up work. Where men are referred to localities where there are not at present facilities for this follow-up work, the association will use its good offices to promote the establishing of such facilities.

In the meantime, the Medical Department of the Army has perfected its machinery for weeding out these tuberculosis cases. Every man passed by the draft board after going into camp is examined by the regimental surgeon, re-examined by a tuberculosis board, and then if suspected of tuberculosis, again examined by a tuberculosis expert. This follows a general policy mapped out and recommended by the association.

A large number of men have already been accepted into the service who were known to be tuberculous, many of them formerly inmates of tuberculosis sanatoria. Part of the association's work has been to get in touch with every tuberculosis sanatorium and dispensary in the country and compile lists of all recent male inmates of draft age, giving the history of their cases and whether or not it was known if they were in the army at present. Hundreds of such names have already been received. This data is forwarded to the training camps, the men are located and the results are reported back to the sources of information.

Furthermore, the association has sent a letter to all of its fifteen hundred local cooperating agencies giving the provisions of the second draft and urging that these agencies procure the names and addresses of all the men of military age in their section who are known to have tuberculosis; get in touch with these men and arm them with the necessary affidavits to prevent, if possible, their being passed by the draft board, and recommend to the local draft boards the names of the approved tuberculosis experts in their section.

The association is also cooperating with the Surgeon-General's office to aid the government in providing sanatoria for those men who have been discharged from the service on account of tuberculosis after their probationary period has expired. All full-fledged soldiers and sailors returned from France or other stations will be cared for as near to their own homes as possible in sanatoria accommodations provided by the government. The government intends to utilize so far as possible existing institutions.

From the United States Marine Corps the association has secured each month a report of men rejected for tuberculosis from all its recruiting stations, and these men will receive the regular follow-up attention.

From the second or educational division of the program it is hoped to de-

rive the greater ultimate good by the establishment of fundamental preventive measures among the well.

The association is interested in any kind of an educational campaign among the men in the various military camps that will tend to promote interest and information with regard to the control and prevention of communicable diseases, and toward the promotion of public and individual health in general. In the mobilization of such large numbers of men in various camps throughout the United States there have developed an unusual number of somewhat serious epidemics of colds, coughs, pneumonia, measles and various other respiratory and communicable diseases. That all of these diseases can be controlled by education and by the exercise of adequate public health measures has been clearly demonstrated in the civilian population throughout the United States. Most of these epidemics are spread through ignorance and carelessness. It is inevitable where large numbers of men from all walks of life and with all possible diseases and variations of physical habits are thrown together in somewhat uncomfortable and crowded living conditions, that there will be an immediate increase in the amount of sickness from communicable diseases. It must be obvious, however, to even the most superficial observer, that if these men can be taught to maintain a reasonable standard of personal hygiene and can be given a knowledge of the methods and principles of the control of communicable diseases a rapid diminution in the sickness rate will follow.

In cooperation with the Educational Committee of the National War Work Council of the Y. M. C. A., the association will furnish a number of stock lectures dealing with tuberculosis together with lantern slides to illustrate them. It will also arrange to put the educational secretaries of each of the camps in touch with public lectures in and around their respective camps. The association has requested the War Department to give careful consideration to the desirability of appointing one or more special officers detailed to lecture on tuberculosis and allied health subjects in all of the army camps throughout the country.

The association has prepared a special circular entitled, "Red Blood," giving in brief and attractive form a message to the soldier relative to personal fitness, a health "Don't Card;" and a Public Health Manual may also be distributed, the latter being a textbook of personal hygiene.

The association will also arrange to distribute through the departmental executives of the Y. M. C. A., a number of special tuberculosis exhibits known popularly as "The Parcel Post Exhibit." In connection with these moving picture films and lantern slides will be used.

The field secretary of the association, Dr. Pattison, is visiting the training camps and supervising this educational work.

—Philip P. Jacobs.

---

### *Reporting of Accidents from Local Anesthetics*

THE Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association has undertaken a study of the accidents following the clinical use of local anesthetics, especially those fol-



lowing ordinary therapeutic doses. It is hoped that this study may lead to a better understanding of the cause of such accidents, and consequently to methods of avoiding them, or, at least, of treating them successfully when they occur.

It is becoming apparent that several of the local anesthetics, if not all of those in general use, are prone to cause death or symptoms of severe poisoning in a small percentage of those cases in which the dose used has been hitherto considered quite safe.

The infrequent occurrence of these accidents and their production by relatively small doses point to a peculiar hypersensitiveness on the part of those in whom the accidents occur. The data necessary for a study of these accidents are at present wholly insufficient, especially since the symptoms described in most of the cases are quite different from those commonly observed in animals even after the administration of toxic, but not fatal, doses.

Such accidents are seldom reported in detail in the medical literature, partly because physicians and dentists fear that they may be held to blame should they report them, partly, perhaps, because they have failed to appreciate the importance of the matter from the standpoint of the protection of the public.

It is evident that a broader view should prevail, and that physicians should be informed regarding the conditions under which such accidents occur in order that they may be avoided. It is also evident that the best protection against such unjust accusations, and the best means of preventing such accidents consist in the publication of careful, detailed records when they have occurred, with the attending circumstances. These should be reported in the medical or dental journals when possible; but when, for any reason, this seems undesirable, a confidential report may be filed with Dr. R. A. Hatcher, 414 East Twenty-Sixth Street, New York City, who has been appointed by the committee to collect this information.

If desired, such reports will be considered strictly confidential so far as the name of the patient and that of the medical attendant are concerned, and such information will be used solely as a means of studying the problem of toxicity of this class of agents, unless permission is given to use the name.

All available facts, both public and private, should be included in these reports, but the following data are especially to be desired in those cases in which more detailed reports can not be made:

The age, sex, and general history of the patient should be given in as great detail as possible. The state of the nervous system appears to be of especial importance. The dosage employed should be stated as accurately as possible, also the concentration of the solution employed, the site of the injection (whether intramuscular, perineural or strictly subcutaneous), and whether applied to the mouth, nose, or other part of the body. The possibility of an injection having been made into a small vein during intramuscular injection or into the gums should be considered. In such cases the action begins almost at once, that is, within a few seconds.

The previous condition of the heart and respiration should be reported if possible; and, of course, the effects of the drug on the heart and respiration, as well as the duration of the symptoms, should be recorded. If antidotes are

employed, their nature and dosage should be stated, together with the character and time of appearance of the effects induced by the antidotes. It is important to state whether antidotes were administered orally, or by subcutaneous, intramuscular, or intravenous injection, and the concentration in which such antidotes were used.

While such detailed information, together with any other available data, are desirable, it is not to be understood that the inability to supply such details should prevent the publication of reports of poisoning, however meager the data, so long as accuracy is observed.

The committee urges on all anesthetists, surgeons, physicians, and dentists the making of such reports as a public duty; it asks that they read this appeal with especial attention to the character of observations desired.

TORALD SOLLMANN, CHAIRMAN

R. A. HATCHER, SPECIAL REFEREE

*Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.*

---

TO THE EDITOR:

In the January number of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE, in my article "War Deafness and Its Prevention—A Report of Tests upon Eight Preventives," the chart on page 230 was inserted through error. It belongs with a later report which will be published in the March issue of the JOURNAL.

—Stacy R. Guild,  
Ann Arbor, Mich.

# *The Journal of Laboratory and Clinical Medicine*

VOL. III.

ST. LOUIS, MARCH, 1918

No. 6

## ORIGINAL ARTICLES

### SUGAR METABOLISM AND DIABETES\*

BY HUGH MCGUIGAN, M.D., CHICAGO, ILL.

THE most interesting thing in medicine is to watch its growth. When a disease is fully understood, its cause known, and its treatment definite, there is little of interest in it. If a patient has malaria, hookworm, or diphtheria, there remains only the routine treatment with quinine, thymol, chenopodium, or antitoxin. The history of the disease and the development of the treatment still retains something of interest, but it lacks the fascination of the research stage. Apart from the purely interesting phase in the development of a disease, the proper perspective can be obtained only by a study of its history and development.

Osler has said that if a person has a knowledge of tuberculosis as developed from the time of Koch, he may have a comparatively good knowledge of the subject, but if he knows nothing of the disease before that time, he still has much to learn about it. This statement places a fair estimate on the study of medical history, and if it be true of tuberculosis, it is doubly true of diabetes. Like tuberculosis, diabetes has been known from the earliest times. Aretæus<sup>1</sup> in the first century left a classic record of the symptoms. Susruta,<sup>2</sup> about the seventh century, knew of the peculiar odor and sweet taste of diabetic urine. Apart from the taste and smell, little more was known until the sixteenth century when Paracelsus<sup>3</sup> (1493-1541), the founder of chemical pharmacology and therapeutics, evaporated a liter (measure) of diabetic urine and found that it contained four ounces of what he called salt, or more than four times the amount found in normal urine. From this discovery, Paracelsus taught that the blood of diabetics contained a salt, which, when eliminated by the kidneys, caused the polyuria. Since the salt in the blood, according to him, was

\*From the Department of Pharmacology and Therapeutics of the University of Illinois. Lecture given before the Graduate School of Medicine.

the cause of the disease, he taught that the treatment should be directed not against the kidney, but toward the cause further back. The kidney, however, had been considered the seat of the disease since the time of Galen,<sup>4</sup> and but little attention was paid to the opinion or teaching of Paracelsus. Whatever we may think of Paracelsus, he, at least, made men think, and better still, made them doubt the traditions and beliefs in medicine that were not founded on fact. It makes little difference what we may think of a man, if he presents facts that can be substantiated, we can not get away from them. If diabetic urine contains four times as much solid matter as normal urine, the fact stands, and must be accepted.

In 1674 Thomas Willis<sup>5</sup> emphasized more strongly than his predecessors the sweet taste of diabetic urine, and Dobson,<sup>6</sup> in 1774, like Paracelsus, evaporated diabetic urine and concluded that the residue was analogous to sugar. Dobson is generally credited with the discovery that diabetic urine contains sugar, but this credit has been protested and he has even been accused of plagiarism on this point. There is no doubt but that at the time others reached the same conclusion. This work of Dobson marks the beginning of the modern era of carbohydrate metabolism and the subject is so closely related to diabetes that the two can not be separated. About the same time, Lavoisier<sup>7</sup> proved that the results of the oxidation of foodstuffs are the same within the body as outside of it and that the body heat is derived from the oxidation of the body substance. In 1815 the French chemist Chevreul<sup>8</sup> demonstrated the identity of grape sugar and diabetic sugar. About this time also Dobson<sup>9</sup> noted that the serum of some diabetics had a sweet taste, and Claude Bernard seventy years later (1847) proved definitely that the blood of diabetics contains sugar.<sup>10</sup> Before this time, however, Tiedemann and Gmelin<sup>11</sup> (1821) discovered that during the digestion of starches in the body, glucose is formed and from this fact they inferred that glucose must be an ingredient of the blood. This inference was confirmed by Magendie and Frerichs in the early forties, several years before Bernard began work, but the work of Bernard on this point is more convincing, and is generally accepted as the first definite proof. Bernard showed the origin of the blood sugar and definitely connected diabetes and carbohydrate metabolism.<sup>12</sup> The term "metabolism" was first used by Liebig<sup>13</sup> in 1842 and is used to include the total chemical changes of foodstuffs under the influence of the enzymes and living cells of the body.

Since we have defined metabolism and almost every one knows, or thinks he knows, what diabetes is, for completeness we might define sugar also. The term itself means something with a sweet taste, and with this as the most characteristic quality, the method of analysis and detection was rather easy in the early days. Unfortunately for this rapid method, it has been discovered that all things with a sweet taste are not sugars, for bodies like glycerin, glycol, saccharin and even lead acetate also have a sweet taste. It is, therefore, quite apparent why some of the early reports of sugar in the blood were not definitely settled until a much later period. Many textbooks on chemistry, while they tell the characters and reactions of sugar, hesitate to give a rigid definition. Time may change the scope of its definition like that of many other chemical bodies. However, for the present, it may be said that a sugar is a carbo-



hydrate that contains one carbonyl group and several hydroxyl groups. One of the hydroxyl groups must be linked directly to a carbon atom in union with the carbonyl group, so that the characteristic group of these compounds is:  $\text{CHOH-CO}$ .<sup>14</sup>

The role of Claude Bernard in the field of sugar metabolism is especially prominent. While he was an intern in the Paris hospitals under Magendie,<sup>15</sup> the therapy of Broussais<sup>16</sup> and Rasori<sup>17</sup> was still in vogue, and although Magendie taught against it, bleeding for almost every disease was a routine treatment. Liters of blood drawn for the therapeutic effect were available to any investigator. Thus it is quite comprehensible why Bernard's thesis for the doctorate was an investigation of the blood sugar. Although Bernard is credited with the discovery of sugar in the normal blood, in his thesis for the doctorate he contended that normal blood did not contain a trace of sugar.<sup>18</sup> It was only after a controversy with the chemist Figuier<sup>19</sup> that he finally proved that sugar is a constituent of normal blood.

His failure at first to detect sugar, when satisfactorily explained, led him directly to another important discovery, that is, the phenomenon of glycolysis or the disappearance of the sugar from the blood on standing. In his first work he used blood that had stood in the hospital wards from twelve to twenty-four hours. When he worked with freshly drawn blood and found that it always contained sugar, he was quick to infer that the sugar in the old blood had disappeared on standing. Investigation easily proved the truth of his conjecture. This phenomenon is of interest in view of the later work of Cohnheim and others on the mechanism of sugar oxidation in the body, and was the foundation of still further research by Bernard.

When the presence of sugar in the normal blood had been established and also the fact that sugar is formed from starches in the gastrointestinal tract, it was quite apparent that the sugar in the blood may be derived directly from the carbohydrates of the food. There still remained to be determined: (1) the changes, if any, that the sugar undergoes during its passage through the intestinal wall; (2) its condition in the blood; (3) the changes through which it passes before oxidation in the body; (4) the difference, if any, between diabetic and normal blood. It is believed by some that the blood sugar is in a combined state or united with something of the nature of an amboceptor and that this amboceptor is lacking in diabetes. This, and other theories have been advanced to explain the increase in the blood sugar in diabetes and the passage of the sugar into the urine.

The most important and fundamental work in sugar metabolism was the discovery of glycogen by Claude Bernard (1857). After many attempts to produce diabetes artificially, he found that puncture or injury of the fourth ventricle caused glycosuria. He then attempted to find the source of the sugar which appeared in the urine, and soon discovered that arterial blood contained more sugar than the venous. When the carotid artery contained 0.12 per cent, the jugular vein contained 0.08 per cent. The relation of the femoral vein and artery was similar. It was evident, therefore, that sugar is lost from the blood to the tissues during the circulation.

With a flexible rubber catheter or sound, he was able to make soundings

and to take blood for analysis from almost any point of the venous circuit.<sup>22</sup> He introduced the sound into the jugular vein, pushed it down into the right heart and found that the venous blood in this region contained as much sugar as the arterial blood. It was clear that somewhere between the femoral vein and the right heart, sugar was added to the blood. To definitely locate the source of this addition, he passed the sound through the heart and down to the level of the kidneys and found that the venous blood at that point contained 0.08 per cent sugar. The sound was then carefully withdrawn to the level of the hepatic vein, where the blood was found to contain 0.14 per cent sugar. In another experiment, he introduced the sound into the crural vein to the level of the hepatic vein and with a syringe, aspirated the blood for analysis. It contained 0.266 per cent, while the inferior vena cava contained 0.088 per cent. By these, and other similar experiments, he proved that the liver is the source of the added sugar.

He also found that if the fresh liver was quickly removed from an animal and thrown into boiling water, only small amounts of sugar were obtained. But there went into solution in the water a substance which made an opalescent solution and which, when boiled with acid, or acted upon by saliva, yielded a reducing sugar. Bernard<sup>23</sup> was also able to isolate and study this substance, which he called *glycogen*. The increased discharge of this substance from the liver is believed to be the commonest cause of most forms of hyperglycemia—at least of those that are transitory. Bernard thought that the increased blood flow through the liver was chiefly responsible for the hyperglycemia, by bringing about an increased rate of the hydrolysis of the glycogen, or glycogenolysis. Others have thought it is due to changes in glycogen formation from sugar, or glycogenesis.

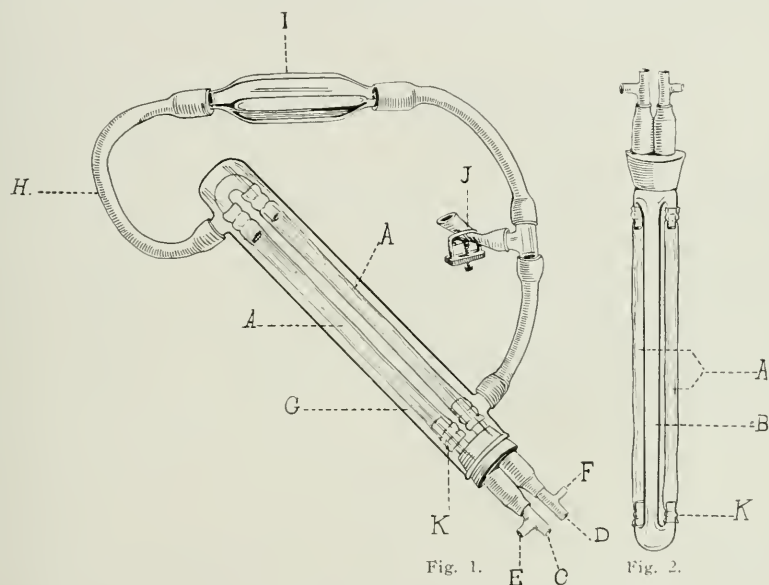
Since the condition of the glycogen in the liver seems to be immediately responsible for the hyperglycemia, it is important to know whether the increased glycogenolysis or decreased glycogenesis is due directly to the liver itself, or whether it is due to defects in other organs which act indirectly on the liver. Almost all other organs have been incriminated in the trouble.

Nicolas and Gueudville, following Rollo,<sup>24</sup> regarded diabetes as a derangement of the digestive functions with the initial lesion in the intestine, and perhaps due to changes in the gastric juice. Although they founded a system of treatment based on this theory, they never attempted to support it by experimental evidence. Recently, Croftan<sup>25</sup> thought that he obtained evidence showing that the intestine is necessary for the polymerization of sugar and the formation of glycogen. According to him the sugar must pass through the intestinal wall, otherwise no glycogen can be formed. He claimed that if the sugar be injected into the veins going directly to the liver, without first passing through the intestine, no glycogen is formed. Pflüger<sup>26</sup> denies Croftan's contention absolutely, and Macleod<sup>27</sup> rejects his evidence as entirely inadequate.

There is also much circumstantial evidence against the intestines being important in the glycogen formation. Karl Grube<sup>28</sup> showed that the liver polymerizes only the monosaccharides. Again, if any of the polymerized forms of sugars, dextrines, or starch are injected into the blood, the diastases of the blood immediately break them down into simple sugars. What is not hydrolyzed

is excreted by the urine or intestine. It would seem, however, that if any polymerization of the sugar from the intestinal tract takes place while passing through the gut wall, it would be hydrolyzed again in the blood, and energy lost in such a mechanism. It is still possible that some other modification of the sugar takes place to aid in glycogen formation, but more evidence is necessary before it deserves serious consideration.

Against this assumption is the condition of the sugar in the blood. The weight of evidence now favors the opinion that the sugar in the blood exists in the same state as it exists in a water solution. The proof of this is as follows: Rona and Michaelis<sup>29</sup> have shown that the proteins of the blood can be precipitated by colloidal ferric hydroxide, and when this is done, all of the sugar is in the filtrate. By this method, the minimal amount of change in the proteins occurs, and suggests strongly that the blood sugar is uncombined. Many



Figs. 1 and 2.—Dialyzing Apparatus Not Requiring Anticoagulants.

1.—Complete apparatus.

2.—Interior part of apparatus.

A—Collodion tubes.

B—Glass support or frame.

C and D—Glass cannula. C is inserted into the artery; D, into the corresponding vein. The side tubes, E and F, are closed with a screw clamp, and rubber, and permit a flushing out of the apparatus without disconnecting, in case of clotting.

When the artery is opened, the blood circulates through the collodion tube A, and is returned to the animal into the vein by D. Water, or saline, surrounds the tubes in the jacket G.

The rubber tubing H and the reservoir I, when raised and lowered, cause the fluid around the tubes to move or circulate, and thus hasten dialysis. The screw clamp, J, permits one to regulate the pressure within the jacket and around the collodion tubes. This is an important procedure and the pressure should be so regulated that the pulsations in the tube resemble those in the artery.

Special precautions should be taken to insure against leaking at the glass and collodion joints K. To secure this, we used adhesive tape and thread ligatures.

others have dialyzed the freshly drawn blood and have been able to dialyze out all of the sugar. If this were a colloidal form, such a separation would be improbable. Even such mild manipulations may break up weak combinations of the sugar with proteins or other colloids, and so to get still nearer the actual condition, C. L. Hess<sup>30</sup> and I dialyzed the normal circulating blood and found that all of the sugar can be dialyzed out. The apparatus which we used was a modification of the dialyzing apparatus used by Abel and his coworkers.<sup>31</sup>

and consists essentially in placing a segment of an artificial artery, composed of collodion, between the cut ends of a normal artery in such a way that it can be immersed in water or saline, and dialysis accomplished while the blood is circulating within the animal. The general technic and the illustration of the instrument will suffice here. Details are given in the *Journal of Pharmacology*, 1914, vi, 45.

The general technic of the experiments was as follows:

Dogs were anesthetized with different anesthetics, and the apparatus attached to the carotid artery and external jugular vein, on the same side, or in the femoral vessels. By avoiding all roughness in the glass and by keeping a pulsation in the dialyzer, we were able to work without an anticoagulant, and by raising and lowering *I*, to cause a circulation of the liquid around the dialyzing tubes, which greatly hastened dialysis. The results obtained by this method are illustrated in Table I.

TABLE I

ANESTHETICS USED	DIALYSIS	PLASMA	Percentage of sugar in		
			DIALYSATE	PLASMA H <sub>2</sub> O	DIALYSATE H <sub>2</sub> O
Morphine	3.0 hrs.	0.135	0.139	0.147	0.141
Morphine	5.0 "	0.125	0.133	0.136	0.135
Morphine	6.0 "	0.088	0.099	0.096	0.100
Ether	4.5 "	0.083	0.087	0.090	0.088
Morphine and ether	5.0 "	0.073	0.082	0.080	0.083
Urethane	2.0 "	0.151	0.168	0.164	0.169
Urethane and ether	3.5 "	0.163	0.175	0.177	0.177
Urethane and ether	4.5 "	0.153	0.169	0.167	0.171
Urethane and ether	1.0 "	0.157	0.173	0.170	0.175

While this work shows that the sugar of the blood is in the same state as in a water solution, there still remains the possibility of a loose combination similar to oxygen and hemoglobin, which might be broken by even such mild manipulations as dialysis. It is difficult to imagine a more delicate method than the dialysis of the normal circulating blood. However, we have tried every method that has suggested a possibility of aiding in the solution of the problem. In comparing different methods of analysis of blood sugar, we found a great variation in the results. It was hard to get a perfect quantitative agreement when different methods were used. Recently some investigators working on the normal level of the blood sugar have come to the conclusion that the higher levels reported are due to excitement. It has been known from the time of Bernard that nervous influences<sup>32, 33</sup> play a great part in regulating the amount of sugar in the blood and that there is some change in the amount of the blood sugar during excitement. We have found that the greatest cause for the variation is in the methods of analysis and especially in the composition of Fehling's solution. The composition of this solution as recommended by various workers varies greatly in alkalinity and we have found that this is the chief cause for the variation reported in the normal level of the blood sugar.<sup>40</sup>

As an indication of the variation in alkalinity of Fehling's solution, the U. S. Pharmacopeia VII recommended 125 grams KOH in 500 c.c. Revision



VIII reduced this to 75 and the current issue IX reduces this to 50 grams. While this difference in alkalinity makes relatively little difference in the yield of sugar from a pure water solution, it makes a great difference in blood analysis. The reason seems to be that the precipitants used to remove proteins leave an interfering body in varying amounts in the filtrate; and in presence of stronger alkali, this body, whatever it may be, prevents the reduction or precipitation of the cuprous oxide.

The two most used methods for the determination of sugar in the blood are the Bertrand<sup>34</sup> modification of Fehling's method and the Lewis-Benedict method.<sup>35</sup> In a recent investigation on the condition of the sugar in the blood, E. L. Ross and I used both of these methods.

Benedict uses picric acid for the removal of the proteins, and the protein-free picric acid filtrate is simply heated with sodium carbonate. In the presence of sugar the picric acid is reduced to picramic acid, which gives a red color. The depth of the color depends on the amount of sugar present, so that the amount can be determined by comparing the color with that of a known solution of sugar.

While engaged in this work, we first noticed the note by W. B. Smith<sup>36</sup> that picric acid does not interfere with the results of Fehling's solution. Such being the case, it renders the two methods directly comparable since the conditions of precipitation can be made exactly alike. The difference, however, between the analyses of the blood-picric filtrate by the two methods was so enormous that we doubted the truth of the statement. We found that in control work, picric acid does not interfere with the Bertrand method, as the following results show: A solution of dextrose was prepared, approximately 0.1 per cent *in water* and determined by the Bertrand method with the following results:

Normal .110%  
.110%

After the addition of 1 gram picric, an amount greatly in excess of saturation, but which went into solution on boiling, the results were:

.113%  
.115%

This slight increase might be considered negligible and within the limits of error. It is constant and due to a salt action, as salts to some extent increase the reduction of alkaline copper.

This influence of salt on the result of the Bertrand method is shown by the following figures:

<i>Sugar in Water</i>		<i>Sugar in Salt</i>		
I	II	III	IV	V
	2% $\text{Na}_2\text{SO}_4$	5% $\text{Na}_2\text{SO}_4$	12% $\text{Na}_2\text{SO}_4$	25% $\text{Na}_2\text{SO}_4$
0.85%	0.88%	0.92%	0.110%	0.110%

Beyond 12 per cent, the influence of the salt does not change. We quote this salt, and 10 to 20 per cent, because hitherto in the precipitation of the proteins from blood, we have used sodium sulphate and acetic acid to a great ex-

tent. The most probable explanation of the salt action is its influence on the boiling point of the solution.

Having convinced ourselves that picric acid does not interfere with the results of the Bertrand method, and that what slight influence it has, is to increase the yield of sugar, we investigated the differences in the results of the Bertrand and the Lewis-Benedict methods. The comparison here is very direct since we use picric acid to remove the proteins in each case, but any method of removal of the proteins of the blood, where acid is avoided, will give results similar to these quoted. We had corroborated the work by the use of (1) alcohol, (2) methyl alcohol, (3) colloidal iron, (4) sodium sulphate, (5) basic lead acetate, (6) dialysis of the serum.

The Lewis-Benedict method was carried out as recommended by Meyers and Bailey.<sup>35</sup> The following table shows some of the results we have obtained by these methods directly, and also by Bertrand's method after "hydrolysis." Details of the work are given in the *Journal of Biological Chemistry*, 1917, xxxi, 553.

Bertrand Direct with 12.5% KOH, Fehling solution	Lewis-Benedict	Bertrand after "hydrolysis" or directly with 5 per cent KOH, Fehling solution
Average.....0.024	0.094	0.099

In seeking for the cause of the great difference between the Bertrand and the Benedict methods, we naturally suspected the presence of a sugar combination—"virtual sugar"—or a sugar of the maltose type, and for this reason hydrolyzed and obtained a rise in the amount, and a figure which in most cases agrees very closely with the Benedict method. There were many facts, however, that made us doubt that there was an actual hydrolysis. First, to get the highest results, we had to use a certain amount of acid, which reduced the alkalinity of Fehling's solution from 12.5 per cent KOH to 5 per cent, and, second, the time necessary for the completion of the supposed hydrolysis was very short, and higher results were obtained if the acid used was not neutralized before applying the Fehling solution. Finally we found if the acid were added directly to the Fehling solution; i. e., lessened alkalinity of the Fehling, the same results were obtained as after the supposed hydrolysis. It is evident, therefore, that lessened alkalinity, and not hydrolysis, is the true explanation. The reason for not neutralizing after hydrolysis and before using the Fehling was due to the fact that there is great variability in the alkalinity of Fehling's solution, as recommended in standard works, and even without neutralizing, we had still a strongly alkaline liquid that corresponded to many so-called Fehling solutions.

With a water solution of dextrose, almost theoretic results can be obtained with Bertrand's method as we have used it. The above results of blood analysis are so striking, and so out of harmony with current opinion, that errors in method, or the presence of interfering bodies, must be seriously considered. Traces of protein or other bodies, which hold cuprous oxide in solution were thought of. The presence of such bodies at first seemed improbable, as is shown by the following work.

First, dextrose added to normal or to diabetic blood can all be recovered.

This has been shown in many cases. Second, the result of dialysis shows that whatever the nature of such a body may be, it is dialyzable. Dialysis does not preclude peptone bodies, which perhaps would be the protein that would interfere most. Such a body, however, would seem to be excluded by the lessening of the alkalinity which removes the effect, and could not remove the interfering body. While the presence of peptone thus seems improbable, its possibility still remains, for we know that traces of peptone beyond detection or recognition markedly influence the Bertrand method, while they have little influence on Benedict's method. If such a body is present its solvent effect is limited, because added sugar can be recovered and changes in the blood sugar are easily recognized. Third, the action of ether: In anesthetized animals, and in diabetics, we can detect no change in the blood protein. Here again the presence of a disturbing body might well escape us, because the amount of sugar in these cases is so large that a change which would make a large percentage change in normal blood would be relatively insignificant here.

Instead of a protein the interfering body might be a dextrin-like body, but the fact that it will dialyze and is easily "hydrolyzed" removes the probability of a dextrin.

Very recently Scott<sup>37</sup> has suggested the presence of a lecithin combination, but this seems improbable since extraction of the picric filtrate or of the blood with ether before precipitation does not change the sugar in any way. This does not disprove Scott's contention that lecithin may combine with dextrose, as he was working under different conditions. It is also not a glycogen-like body because when alcohol is used to precipitate the proteins, the glycogen would be removed, and yet we obtain results *similar* to the picric acid method.

The only investigator, so far as we know, who has reported the normal level of the blood sugar nearly as low as the figures we give for the direct determination after precipitation by picric acid is Shaffer.<sup>38</sup> His average for the normal blood sugar in four dogs was 0.036, 0.020, 0.046, and 0.026 per cent, figures that agree closely with ours by the direct picric acid method. His results after anesthesia show a considerable rise, showing also, we think, agreement with our explanation that sugar added by any cause can be recovered. The low results he at first obtained we think are due to the solvent action of the strong alkali on the cuprous oxide which is increased by a yet unknown body in the blood. This solvent action is fully satisfied by the amount of sugar in the normal blood so that added sugar or the increase due to anesthesia may be recovered. This solvent action is much greater in a blood filtrate than in a water solution. If the picric filtrate is boiled alone or if sodium sulphate is added and it is boiled, a higher sugar yield is obtained. This seems to be due to the removal of an interfering body.

Anesthesia and morphine increase the amount of sugar when determined by the Bertrand method after precipitation of the proteins by picric acid. This action is apparently not due to the breaking up of a sugar combination, because blood extracted directly with ether is not changed, nor is picric blood filtrate changed by similar treatment.

That morphine increases the sugar as determined by this method is shown by the following experiment.

	Fehling Solution	
	12.5% KOH	5% KOH
Normal dog. Blood sugar direct	0.015%	0.08%
3 hrs. after 0.15 gm. morphine	0.165%	0.225%
Normal dog	0.020%	0.110%
After ether	0.080%	0.156%

The opinion has been expressed by more than one writer that the sugar in the blood exists in combination with something of the nature of an amboceptor.<sup>39</sup> There are some facts brought out by the picric acid precipitation of proteins that might be used to sustain this theory. Since the use of picric acid seems to show that the blood sugar is not a simple solution of glucose in water, its use also permits the testing of the amboceptor hypothesis and to a considerable extent supports that theory.

For example, Shaffer<sup>38</sup> found that dextrose added to a solution of sugar that had fermented with yeast could not be completely recovered. We have been able to confirm this statement, and since the utilization of the dextrose by the yeast cell is probably by the same mechanism as in the animal cell, this masking or combining with the sugar, suggests a similar preliminary step in the animal body. Accordingly, we have tested blood by:

1. Adding sugar directly to it when fresh, and determining the amount of the added dextrose that could be recovered.
2. The same addition after blood had undergone glycolysis.
3. Comparing normal and diabetic blood.

In the normal blood the *added* sugar can be recovered, while in the blood that had been freed from sugar by glycolysis, added sugar is immediately masked as in yeast fermentation, and can not be recovered until after the addition of acid. This suggests that the blood sugar normally is in combination, and that when the sugar is removed by glycolysis, the amboceptor<sup>39</sup> still retains the power to unite with free dextrose, converting it into the same form as the dextrose normally existing in the blood.

In like manner we determined the sugar in *normal* blood directly by the picric acid Bertrand method, and found that all the added sugar can be recovered, while in blood that had undergone glycolysis, a certain amount apparently combined with the amboceptor, and could be recovered only after adding acid or reducing the alkalinity of Fehling's solution. The following experiments illustrate this.

Normal blood was glycolyzed until it showed no sugar when tested with Fehling's solution.<sup>40</sup> The Benedict method showed 0.016%; Fehling containing 2.5 per cent NaOH or 10 per cent Na<sub>2</sub>CO<sub>3</sub> gave 0.01 per cent. If, now, 100 c.c. of this glycolyzed blood be precipitated with picric acid and to the picric acid filtrate we add 10 c.c. of dextrose solution—or 0.102 per cent—and determine how much of this can be recovered by varying the alkalinity of Fehling's solution, we find for the original undiluted Fehling containing 12.5 per cent NaOH—only 60 per cent recovered, or 0.060. Five per cent NaOH gives 0.110; 2.5 per cent gives 0.120 per cent. We thus find that added sugar can be recovered if not too much alkali is added, or if sodium carbonate is used instead of KOH.

Controls with water solution gave with 12.5 per cent NaOH, 0.102 per



cent; with 5 per cent NaOH, 0.110 per cent. With the weaker alkali, consequently, we recover the total sugar added, and the actual or total sugar of the blood.

The following results show how changing the alkalinity influences the yield of the sugar as determined by Bertrand's modification of Fehling's solution.

NaOH% in Fehling's sol.	Dog's blood sugar
12.5%	0.018%
5 %	0.090%
4 %	0.105%
2.5%	0.122%

The Lewis-Benedict method on this blood gave 0.106 per cent.

Increasing the amount of copper in the Fehling solution while the alkalinity remains constant (12.5% NaOH) has the same effect as decreasing the alkalinity and keeping the amount of copper sulphate constant, as the following analysis of dog's blood will show.

CuSO <sub>4</sub>	Per cent sugar
2%	0.021%
4%	0.044%
8%	0.096%

A solution of dextrose in water or in picric acid solution is not nearly so much influenced as a blood filtrate by the variation of the copper, or by changes in the alkalinity. The following table shows the relatively slight variation of the same dextrose solution in water by varying the copper in Fehling's solution, while the alkalinity remains 12.5% NaOH.

CuSO <sub>4</sub>	Per cent dextrose
2%	0.070%
4%	0.088%
8%	0.102%

#### THE DIFFERENCE BETWEEN NORMAL AND DIABETIC BLOOD

We have examined the blood of several normal and diabetic persons which, at one stage of the work, we thought showed a significant difference. When examined more closely, we think no such difference exists. The ratio of the amount of sugar found by using 12.5 per cent KOH in Fehling's solution to that determined by using 5 per cent KOH Fehling is striking.

##### *Ratio in Normal Dogs*

12.5% of KOH Fehling	5% KOH Fehling	Ratio
0.048%	0.140%	1:3
0.010%	0.067%	1:7
0.028%	0.070%	1:2.5
0.025%	0.092%	1:4
0.020%	0.110%	1:5
Average of 12		1:4

The influence of anesthesia, as is well known, raises the blood sugar, and if continued long enough, may cause a glycosuria. It also changes this ratio remarkably.

	12.5% KOH	5% KOH	Ratio
I. Normal	0.020%	0.110%	1:5
After 2 hours ether anesthesia	0.08 %	0.156%	1:2
II. After 10 minutes ether	0.052%	0.104%	1:2
III. After 3 hours ether	0.205%	0.260%	1:1.5

Ether always reduces this ratio, as does anything that raises the amount of sugar in the blood. In six dogs under ether for 1 hour, the ratio fell from 1:4 to 1:3. In a case of adrenaline glycosuria without ether, the ratio was practically 1:1. In one case of phlorizin diabetes, in which, as is well known, the blood sugar does not increase, the ratio did not change.

In normal human beings, the ratio is practically the same as in normal dogs.

No.	12.5% KOH	5% KOH	Ratio
1	0.025%	0.090%	1:3.6
2	0.035%	0.160%	1:4.8
3	0.082%	0.230%	1:3
4	0.082%	0.240%	1:3

Human diabetic blood again shows almost a ratio of 1:1 as the following results from 3 cases of diabetes will show.

No.	12.5% KOH	5% KOH	Ratio
1	0.172%	0.256%	1:1.5
2	0.290%	0.307%	1:1.05
3	0.670%	0.730%	1:1.1

Some of this diabetic blood was allowed to glycolyze in the incubator at 40° C. until the sugar had all disappeared, and then a known amount of glucose was added. Of the added glucose, in two cases only, 40 and 60 per cent were recovered, when analyzed by the strong alkali Fehling, so that if there be such a thing as an amboceptor, it acts, so far as we can make out, as strongly in diabetic blood as in normal.

Closer analysis leads us to reject the amboceptor theory and to incline towards the opinion that there is no difference in the state of the sugar in normal and diabetic blood. The difference in the amount of blood sugar found when we use a Fehling solution which contains 12.5% NaOH and the amount found when we use 5% NaOH Fehling is enormous. The difference between the results obtained by the stronger Fehling solution and the Lewis-Benedict is about the same as between the two concentrations of the Fehling solution. When all these methods are tested on a solution of dextrose in water, there is very little difference in the results obtained by all of these methods. Consequently, at first one is inclined to believe that the sugar in the blood exists in a different state than sugar in water. The results obtained from a blood filtrate suggest at first that some of the blood sugar is in a combined form. The substance to which the sugar may be combined might be considered as the postulated amboceptor. On analysis, however, it is seen that the difference in the results of these methods depends on an interfering body. This body apparently interferes more in strong alkali than in weaker, and it is just as active in the blood of pronounced diabetes as in the normal blood. It does not interfere with the Lewis-Benedict method.

In the worst case of diabetes cited, the blood sugar found by using 12.5%

NaOH Fehling solution was 0.670% while that found by using 5% NaOH was 0.730%. The ratio here is approximately 1:1. This might mean that no amboceptor was present. The actual difference in the amount of sugar in this case as determined by the two concentrations of Fehling's solution is 0.06% or exactly the amount of difference in normal blood. On account of the low initial figure of normal blood, the ratio would be about 1:4. Consequently we are forced to admit that this ratio does not mean that there is a difference between diabetic blood and normal blood.

It has been shown above that after glycolysis diabetic blood will act towards added sugar just as normal blood, and the reason the added sugar can not be recovered by the Bertrand method is because of an interfering body.

The nature of this body is organic because blood ash does not interfere. Alkali normally dissolves some cuprous oxide. In the presence of this organic substance which picric acid does not remove, the solvent action of the strong alkali is increased. Looked at in this way, there is nothing to indicate an amboceptor or sugar in the blood in a combined form.

Since direct examination shows that the sugar of the blood exists as a water solution, we have sought by indirect methods to gain further evidence either for or against this opinion. One method of attack was to determine whether or not tissues or cells can utilize free sugar.

Ludwig<sup>41</sup> thought that oxidation took place in the blood. We now know that the blood sugar is oxidized by the form elements of the muscles and glands mainly, and only in minute amount in the blood. No oxidation takes place in the serum other than that due to the alkalinity of the solution. I have kept serum for ten days in the incubator at 40° C., without an appreciable loss of sugar, while in the whole blood, the sugar under the same conditions disappears completely in twenty-four hours. When the blood is laked, glycolysis stops. The corpuscles contain very little, if any, sugar. All these facts are in keeping with the opinion that the blood sugar is in free condition. That the oxidation takes place in the form elements, and that the free sugar can be directly oxidized, is supported by many other facts. First, in fermentation of yeast, there is no action outside the cell, no extracellular enzyme. This may be easily shown by adding yeast to a solution of dextrose, and, when fermentation is progressing rapidly, by filtering the solution through an ordinary filter paper. Filtration by straining out the yeast cells stops fermentation instantly. Again, blood flowing through living tissue loses some of its sugar, as Bernard has shown. The amount is small, but may be increased by muscular contraction; in fact, this is a method sometimes used to free an animal of glycogen. By use of strychnine tetanus, strong muscular contractions, or other means leading to extreme fatigue, the liver becomes glycogen-free. That the free sugar can be used in this way is shown by perfusing isolated organs, as the heart, or muscles. Years ago, I perfused the isolated hind legs of animals, keeping one leg in each case for control, and while the perfusion was proceeding, kept the muscles working by electrical stimulation. The results clearly showed a loss of sugar, apparently by direct utilization. This work was done when we believed that the blood sugar existed in a colloidal state. By more refined methods, more exact figures may be obtained, but we think that the facts will remain essentially the same.<sup>42</sup>

The method used consisted in perfusing the organ under investigation with blood containing a known amount of sugar, and determining the loss of sugar during the perfusion. One hind leg of an animal was perfused and the other used as control. Account was taken of the amount of glycolysis that the blood would have undergone during the time of the perfusion, the changes in the glycogen content of the leg, the changes in the sugar content of the leg, and the loss of sugar from the blood. While the perfusion lasted, the leg was stimulated through the nerve with an electric current. The idea of this was to increase the amount of sugar oxidized.

During the perfusion there was never found any formation of glycogen. *Apparently the power of glycogen formation is lost before the power to oxidize sugars.* This point would indicate that glycogenesis may be most at fault in diabetes. The monosaccharides only were oxidized. When the leg failed to respond to electrical stimulation, the power to oxidize sugars was greatly reduced and even lost. Apparently the living protoplasm is the active agent. A few of the results are presented calculated for 100 gm. muscle:

Sugar used	Time of perfusion	Amount of sugar used
Dextrose	60 min.	15 mg.
Levulose	60 "	25 "
Levulose	60 "	33 "
Galactose	60 "	81 "

The heart while contracting also uses sugar. This has been shown by Knowlton and Starling,<sup>43</sup> by Rhode<sup>44</sup> and by Locke and Rosenheim.<sup>45</sup> Camis,<sup>46</sup> however, claims that it may not have been the perfused sugar that was directly oxidized, but the stored glycogen of the heart itself. There seems little basis for such contention. Our own work shows that the sugar may be used directly by the contracting muscle.

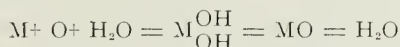
The sugars that are used directly are those that are directly fermentable by yeast. The products are the same in both cases, and I believe the mechanism is also the same. In each case the living cell is the active agent. This can be readily shown in the case of yeast by simply passing through ordinary filter paper a solution of sugar that is undergoing rapid fermentation. The filtrate at once ceases to evolve gas. It is true, as Buchner<sup>47</sup> has shown, that when the yeast cell is ground up the filtered juice will still cause fermentation, but the significance of this fact has been much overestimated, and the time that such fermentation will proceed is very short indeed, and suggests that it may be due to living particles of the cell protoplasm. Similar, but more marked, is the depressing influence of disintegration of animal tissues on the oxidative properties. This, I think, was the cause of Cohnheim's<sup>48</sup> failure to demonstrate the synergistic action of muscle and pancreas extracts on the oxidation of glucose. There can be no doubt about the interrelated action of the pancreas and muscle in the normal animal, but since it is the living cell that oxidizes glucose, or at least the enzyme in the living cell, we should not expect in all cases that the same action can be demonstrated when the cell is disintegrated. The conjecture was legitimate when isolated enzymes were considered as the sole source of oxidation. The method of Knowlton and Starling<sup>49</sup> on the contracting heart,



or of Macleod<sup>50</sup> on the eviscerated animal, seems a better method of attacking this problem.

Lavoisier demonstrated that the results of oxidation of foodstuffs in the body is the same as oxidation out of it. Sugar oxidized in a calorimeter or in the body, produces the same number of calories or heat energy, and inside the body this heat is available for the body. This does not mean that the mechanism of the oxidation is the same in the two cases. As a matter of fact, we do not know how such simple oxidations as the burning of carbohydrate or magnesium take place. We know that as the result of such oxidation, heat is liberated; but as to how it is transferred to the body, we are still in the dark.

This is one point in which the two types of oxidation may be the same. Moritz Traube<sup>51</sup> considers that the simplest oxidation is an hydrolysis, that is, oxidation will not take place in the absence of water. This may be represented by the following formulæ:



and the water can again be used in the process. The oxidations in the body are probably also hydrolytic, but must be more complicated in nature than the simple burning of a magnesium ribbon, or a splinter of wood in the air. It is generally accepted that foodstuffs burn, and are used by the body only after they have become an actual part of the body. In other words, it is the living matter of the body that burns. It is the disintegration of the food molecules after they have become a part of the biogen or living nucleus that renders the body such an economic machine. The rupture of the carbohydrate is an exothermal reaction, and the energy when liberated is already a part of the machine. Concerning what the living molecule or biogen is—if such exist—no satisfactory opinion has yet been advanced. The characters of mitochondria suggest that they may be of fundamental importance,<sup>52</sup> and approach the characters of the hypothecated biogen.

Since the greater part of the digested carbohydrate is changed to glycogen in the body, and this again to glucose before utilization by the tissues, it is of interest to know what conditions modify glycogenolysis. As is well known, some agents increase the blood sugar while others reduce it. I wish only to mention a few of these which we have recently investigated. In the first place, what is the effect on the blood sugar of modifying the circulation through the liver? Bernard<sup>53</sup> thought increased liver circulation was the cause of diabetes. E. L. Ross and I,<sup>54</sup> accordingly, studied the effects on the blood sugar of (1) ligation of the portal vein, (2) ligation of hepatic artery, (3) simultaneous ligation of both portal and hepatic, (4) hyperarterialization and, (5) Eck fistula and, (6) reversed Eck fistula.

The results are shown in the accompanying tables. Hyperarterialization of the liver was accomplished by first making an Eck fistula and then turning an artery into the portal vein or its branches. The results were obtained from acute experiments of one hour duration, and clearly show that ligation of the portal vein, or greatly increasing the arterial blood going through the liver, are the only operations to cause any marked change in the blood sugar, and these changes are never sufficient to cause glycosuria. Eck fistula and the reverse,

also in animals that were kept for months by Robert Keeton,<sup>55</sup> had little influence on the blood sugar. We hold, therefore, that no conceivable change in the circulation can be of much importance as a causation of diabetes, unless the change secondarily involves the pancreas or some other organ. It is well known that if circulatory changes involve the pancreas sufficiently to produce inflammation of that organ, glycosuria may result. Uncomplicated changes in the liver circulation can be of only small import as a direct factor. This is of interest, in view of the fact that Bernard thought pique, or puncture of the fourth ventricle acted to cause glycosuria by changing the liver circulation. It is now known that unless the adrenals and liver are both intact, puncture alone will not cause glycosuria.

The average of the results of modifying the circulation are:

No. of Expts.	Percentage of Dextrose in Blood		Length of time ligated	Actual sugar increase
	Before ligation	After ligation		
Portal ligation 8	.088%	.145%	59 min.	0.056%
Hepatic ligation 8	.105%	.124%	60 "	0.019%
Portal and hepatic 8	.126%	.114%	50 "	-0.012%
Hyperarterialization	.176%	.238%	50 "	0.062%
Hypervenosity 5	.141%	.157%	60 "	0.016%

#### Summary Table

	Increase calculated by difference in percentage before and after operation	Increase expressed in per cent of sugar content before operation
Ligated portal	0.056%	62%
Ligated hepatic	0.019%	18%
Portal and Hepatic	0.012%	- 9%
Hyperarterialization	0.062%	35%
Hypervenosity	0.016%	11%

#### CONDITIONS REDUCING THE BLOOD SUGAR

Hypoglycemia has not been considered so important as hyperglycemia. The reason is very apparent. Diabetes is so common and so fatal a disease that all phases of it have attracted attention. When, therefore, it was found that the blood sugar is usually increased and that when it reaches a certain concentration, it passes into the urine, the cause of the disease was thought to be closely associated with the blood. On the other hand, we know of no disease where hypoglycemia is considered so important as the hyperglycemia of diabetes. The methods which reduce the blood sugar are worthy of attention for the following reasons: First, they may illuminate the process of normal sugar metabolism; and, second, they may reveal something of value in treatment. While such possibilities exist, it must be confessed that all of the known methods of blood sugar reduction, with the exception of Allen's method<sup>56</sup> are of much more immediate injury to the animal than the most rapidly progressing hyperglycemia.

The conditions that may cause a reduction of the normal sugar concentra-

tion are: extreme exhaustion,<sup>57</sup> adrenal insufficiency,<sup>58</sup> Addison's disease,<sup>59</sup> thyroid insufficiency,<sup>60</sup> poisoning by phosphorus,<sup>61</sup> and hydrazine<sup>62</sup> or phlorizin,<sup>63</sup> high section of the cord,<sup>64</sup> anaphylactic shock,<sup>65</sup> intercurrent affections,<sup>66</sup> moribund states,<sup>67</sup> and the injection of foreign proteins.<sup>65</sup> The striking physiologic changes that result from the application of most of the methods are: low blood pressure, splanchnic dilation, depleted vitality, and, in larger doses, a moribund state. In none of them is there the remotest suggestion of anything that operates for the welfare of the organism. Various artificial hyperglycemias and glycosurias have been reported to be lessened by the administration of certain glandular extracts or salt solutions. Stenström<sup>68</sup> found that pituitrin lessened adrenalin hyperglycemia. Dresel<sup>69</sup> reports that extracts of the pituitary, thyroid, ovary, or pancreas, when injected with epinephrin reduce the height of the hyperglycemia. Miculicich<sup>70</sup> found that hirudin inhibits, and Glaessner and Pick<sup>71</sup> found that pancreatic juice and Witte's peptone lessen epinephrin glycosuria. Bock and Hoffman<sup>72</sup> showed that salt glycosuria is lessened by the further action of the injected saline. It is now known that calcium chloride will lessen salt glycosuria. Underhill<sup>73</sup> found that the glycosuria produced by morphine, pyridine, etc., is reduced by the free administration of oxygen. Other cases of the kind are reported by Allen.<sup>74</sup> It should be stated, however, that the whole subject is unsatisfactory and contradictory to such an extent that many substances are recorded by some to produce hyperglycemia or glycosuria, while according to others the same drug causes hypoglycemia. Phosphorus, salt solutions, and peptone are among such drugs. Also, in most moribund states the blood sugar increases immediately before death.

In making blood transfusions from one normal animal to another, we have noticed that there is a general tendency for the blood sugar of the recipient to fall. We have since found that this is a common result of the intravenous injection of proteins, and especially of peptones, although Henderson and Underhill<sup>75</sup> have reported glycosuria following the injection of peptone. We can not confirm their results; and look upon the glycosuria they describe, not as a peptone, but as either an ether glycosuria or as an exception to the regular peptone action. Similar exceptions may be seen in those cases in which peptone hastens, rather than retards the coagulation of the blood.<sup>76</sup> The whole subject of the action of sera, organ extracts, and toxins on the sugar content of the blood is in an unsatisfactory state, and the action perhaps varies with conditions.

In our work we used Witte's peptone chiefly but we obtained similar results with silk peptone, and with gelatin; and in several cases of anaphylactic shock, which resembles peptone action, we found a hypoglycemia.

For the study of peptone hypoglycemia we used dogs entirely. The number of animals used without anesthesia was thirty. The average weight was 10.6 kilos and ranged from 4 to 18.5 kilos. The average dose of peptone, which was 3.7 grams, was given intravenously in about 20 c.c. of water. The average normal blood sugar content was 0.65 per cent. In from two to five hours after the injection of peptone, the blood was again analyzed. On the average, the time was three and one-half hours. The blood sugar by this time on the average, was reduced to about one-half the original content, or 38 per cent. We found a great reduction also when an anesthetic was used. The cause of the reduction

is not clear. Perhaps it is due to changed circulation through the liver, although this can not be sustained by experiment. It may also be due to a paralysis of the normal mechanism of glycogenolysis. The liver still contains considerable quantities of glycogen. In three cases which we analyzed, the glycogen content of the fresh liver was 0.45 per cent, 1.91 per cent and 3.18 per cent. The blood sugar in the last case was lower than any of the others. It may be that there is a greater utilization of the sugar in the circulating blood and lessened glycogenolysis, but this we have not investigated. The most common changes after peptone injections are low blood pressure and the unsatisfactorily explained condition known as extreme fatigue, or shock. None of the measures that reduce the blood sugar by the use of drugs offers the slightest hope for the treatment of diabetes. When, however, we reflect that almost all the definite knowledge we have regarding diabetes is the product of less than one hundred years of research, there is much to be hoped from a continuation of the study.

## BIBLIOGRAPHY

- <sup>1</sup>Bernard: *Le Diabete et la Glycogenese Animale*, Paris, 1877, pp. 57, 145.
- <sup>2</sup>Lusk: *Elements of Nutrition*, W. B. Saunders Co., Philadelphia, 1909, p. 271.
- <sup>3</sup>Lepine: *Le Diabete*; Felix Alcan, Editor, Paris, 1909, 2. Garrison: *History of Medicine*, W. B. Saunders Co., Philadelphia, 1914, p. 139.
- <sup>4</sup>Lepine: *Loc. cit.*, 1. Bernard: *Loc. cit.*, 145. Galen Lib. XXVI. De Locis mal Affectis, Cap. III.
- <sup>5</sup>Bernard: *Loc. cit.*, pp. 58, 145.
- <sup>6</sup>Dobson: *Medical Observations and Inquiries by a Society of Physicians in London*, pub. 1776, v, 298-316, but reported in 1874.
- <sup>7</sup>Rubner: *Ztschr. f. Biologie*, 1893, xxx, 73-142.
- <sup>8</sup>Chevreul: *Annales de Chemie et de Physique*, Paris, 1815, pp. 95-319.
- <sup>9</sup>Dobson: *Loc. cit.*
- <sup>10</sup>Bernard: *Loc. cit.*, p. 127.
- <sup>11</sup>Tiedemann and Gmelin: *Die Verdauung nach Versuchen*, Heidelberg and Leipzig, 1826, i, 184.
- <sup>12</sup>Bernard: *La Diabete et la Glycogenese Animale*, 1877, p. 159. Original: *Compt. rend. Acad. d. sc.*, July 27, 1846, xxiii, 187.
- <sup>13</sup>Die Organische Chemie in ihrer Anwendung auf Physiologie und Pathologie, Braunschweig, 1842, p. 9.
- <sup>14</sup>Holleman-Walker: *Textbook of Organic Chemistry*, John Wiley & Sons, New York, 1910, p. 255.
- <sup>15</sup>Bernard: *Loc. cit.*, p. 226.
- <sup>16</sup>Garrison: *History of Medicine*, 1914, p. 139.
- <sup>17</sup>Garrison: *Loc. cit.*
- <sup>18</sup>Lepine: *Le Diabete*, pp. 12, 13.
- <sup>19</sup>Figuier: *Memoire sur l'origine du sucre contenu dans le foie*, *Gaz. hebd. de med.*, 1855, p. 83; *Ibid.*, p. 236; Quoted by Lepine: *Loc. cit.*, p. 18.
- <sup>20</sup>Bernard: *Loc. cit.*, p. 207.
- <sup>21</sup>*Le Diabete et la Glycogenese Animale*, *Compt. rend. Acad. d. sc.*, 1857, xlv, 578, p. 162 ff.
- <sup>22</sup>Bernard: *Leçons de physiologie experm. Appliquee a la medicine*, Paris, 1855, 1, 288. *Leçons sur le systems nerveux*, Paris, 1858, i, p. 397; ii, 528.
- <sup>23</sup>Bernard: *Le Diabete et la Glycogenese Animale*, pp. 281, 288. Also Lepine: *La Diabete*, p. 25.
- <sup>24</sup>Rollo: Quoted by Bernard: *Ibid.*, pp. 60, 76.
- <sup>25</sup>Croftan: *Arch. f. d. ges. Physiol.*, 1909, cxxvi, 407.
- <sup>26</sup>*Ibid.*, p. 416.
- <sup>27</sup>MacLeod: *Diabetes, Its Pathology*, New York, Longmans, Greene & Co., 1913, p. 55.
- <sup>28</sup>Grube: *Arch. f. d. ges. Physiol.*, 1907, cxxviii, 1.
- <sup>29</sup>*Biochem. Ztschr.*, 1908, vii, 329; *Ibid.*, 1909, xvi, 60.
- <sup>30</sup>Hess and McGuigan: *Jour. Pharmacol. and Exper. Therap.*, 1914, vi, 45.
- <sup>31</sup>Abel: *Ibid.*, 1914, v, 275.
- <sup>32</sup>Scott: *Am. Jour. Physiol.*, 1914, xxiv, 271.
- <sup>33</sup>Shaffer: *Jour. Biol. Chem.*, 1914, xix, 285, 297.



- <sup>34</sup>Bertrand: Bull. de la Soc. Chem. de Paris., 1906, xxxv, 1285. Moeckel and Frank: Ztschr., f. physiol. Chem., 1910, lxx, 323.
- <sup>35</sup>Lewis and Benedict: Jour. Biol. Chem., 1915, xx, 61. Meyers and Bailey: Ibid., 1916, p. 147.
- <sup>36</sup>Smith, W. B.: Science, Aug. 11, 1916, n. s. xlv, 213.
- <sup>37</sup>Scott, E. L.: Am. Jour. Physiol., March, 1916, xl, No. 1. Proc. Soc. Exper. Biol. and Med., 1917, xiv, 34.
- <sup>38</sup>Shaffer: Loc. cit.
- <sup>39</sup>Allen: Glycosuria and Diabetes, 1913, pp. 382, 383, *et passim*.
- <sup>40</sup>McGuigan and Ross: Jour. Biol. Chem., 1917, xxxi, 533.
- <sup>41</sup>Lusk: Elements of Nutrition, W. B. Saunders Co., Philadelphia, 1909, p. 19.
- <sup>42</sup>Am. Jour. Physiol., 1908, xxi, 334.
- <sup>43</sup>Knowlton and Starling: Proc. Roy. Soc., 1912, lxxxv, 218.
- <sup>44</sup>Rhode: Ztschr. f. physiol. Chem., 1910, lxxviii, 181.
- <sup>45</sup>Locke and Rosenheim: Jour. Physiol., xxxvi, 205.
- <sup>46</sup>Camis: Ztschr. f. Allg. Physiol., 1908, viii, p. 371.
- <sup>47</sup>Buchner: Chem. Berichte, 1897-1898, xxx, pp. 117, 1110, 2268, 31, 32.
- <sup>48</sup>Cohnheim: Ztschr. f. physiol. Chem., 1903, xxxix, 336; Ibid., 1904, xlii, 401; Ibid., 1905, xliii, 547; Ibid., 1906, xlvi, 253. McGuigan: Am. Jour. Physiol., 1908, xxi, 351. McGuigan and Hess: Ibid., 1912, xxx, 341.
- <sup>49</sup>Knowlton and Starling: Loc. cit.
- <sup>50</sup>Macleod and Pearce: Am. Jour. Physiol., 1913, xxxiii, 184.
- <sup>51</sup>Traube: Ber. d. deutsch. chem. Gesellsch., 1882, xv, 666.
- <sup>52</sup>Cowdry: Am. Jour. Anat., 1916, xix, 423; Ibid., 1914, xvii, 1. International Monatschrift f. Anat. u. Physiol., 1914, 31, 267.
- <sup>53</sup>Bernard: Loc. cit., piqure.
- <sup>54</sup>McGuigan and Ross: Am. Jour. Physiol., 1916, xxxix, 480.
- <sup>55</sup>Unpublished experiments.
- <sup>56</sup>Allen: Boston Med. and Surg. Jour., 1915, clxxii, 693-730.
- <sup>57</sup>Weiland, W.: Deutsch. Arch. f. klin. med., 1907-1908.
- <sup>58</sup>Mayer: Comp. rend. Soc. de biol., 1906, lxiv, 219.
- <sup>59</sup>Porges, O.: Ztschr. f. klin. Med., 1910, lxix, 243.
- <sup>60</sup>Cushing, H.: The Pituitary Body and its Disorders, J. B. Lippincott Co., Philadelphia, 1910, pp. 132, 262.
- <sup>61</sup>Frank E., and Isaac, S.: Arch. f. exper. Path. u. Pharmacol., 1911, lxiv, 274.
- <sup>62</sup>Underhill, F. C.: Jour. Biol. Chem., 1911-12, x, 159.
- <sup>63</sup>Von Mering: Phloridzin, Ztschr. f. klin. Med., 1888, xiv, 405; Ibid., 1889, xvi, 431.
- <sup>64</sup>Bernard: Leçons sur la Physiologie et la Pathologie du système nerveuse Pans., 1858, i, 466, 482. Chaveau, A., and Kaufmann, M.: Compt. rend. Acad. d. sc., 1893, cxvi, 298, 551, 613.
- <sup>65</sup>McGuigan and Ross: Jour. Biol. Chem., 1915, xxii, 417.
- <sup>66</sup>Cambridge: Glycosuria and Allied Conditions, London, 1913, p. 180.
- <sup>67</sup>Macleod: Loc. cit., p. 58. Bock, C., and Hoffman, F. A.: Jahresb. f. Thier Chem., 1874, iv, 440.
- <sup>68</sup>Stenström: Biochem. Ztschr., 1913-1914, lviii, 472.
- <sup>69</sup>Deisel: Ztschr. exper. Path. u. Therap., 1914, xvi, 365.
- <sup>70</sup>Miculicich: Arch. f. exper. Path. u. Pharmacol., 1912, lxix, 128.
- <sup>71</sup>Ztschr. f. exper. Path. u. Therap., 1909, vi, 313.
- <sup>72</sup>Reichert and Du Bois Reymond: Arch. f. Anat., Physiol. u. Wissensch. Med., 1871.
- <sup>73</sup>Underhill, F. P.: Jour. Biol. Chem., 1905-1906, i, 113.
- <sup>74</sup>Allen: Glycosuria and Diabetes, 1913, p. 855 ff.
- <sup>75</sup>Henderson: Yandell, and Underhill, F. P.: Am. Jour. Physiol., 1911, p. 280.
- <sup>76</sup>Stewart: Manual of Physiology, ed. 5, 1906, W. B. Saunders Co., Philadelphia, p. 280.

# WAR DEAFNESS AND ITS PREVENTION—A REPORT OF FURTHER TESTS UPON PREVENTIVES\*

BY STACY R. GUILD, ANN ARBOR, MICH.

## METHOD OF TESTING

THE tests here reported were made by means of the tambour method. Using the rubber ear previously described to hold the various preventive devices, connection was made from the "medial" end of the canal to a tambour and the records of the amount of the detonations that passed the preventives were made on smoked paper instead of as physiologic reactions in animals' ears. The guns used as sources of the detonation waves were clamped in a constant position, therefore the relative amount of force that passed the various devices is indicated by the height to which the tracer of the tambour moved. Blank cartridges were used; these vary considerably, due probably to the packing of the wadding. But the average of several shots gives fairly uniform results, so it has not been checked up with ball cartridges, which would probably be more uniform in the individual loads.

## DETAILS OF ARRANGEMENT OF APPARATUS

The rubber ear was placed in the wall between two rooms which are connected by a third room. The wall is an ordinary lath and plaster wall, with an opening 12 by 24 inches in it. This opening was closed by a board one inch thick in which a hole two inches in diameter was drilled. The rubber ear was fastened into a piece of board one inch thick and six inches square by drilling a hole in the center of the piece and then sawing it across the hole and carefully trimming away parts until the ear fits properly into the opening. The halves were firmly fastened together by strips screwed on, and the whole piece, containing the ear, was fastened firmly onto the board partition with the ear in the two-inch opening. This opening was beveled so as to be about four inches in diameter at the surface of the board. It was feared that this wall would permit sufficient shock to pass to affect the tambour by acting on the upper surface of the membrane, and that it would be necessary to resort to another location after preliminary tests, but such action on the tambour was not observed.

The tambour that was found to be suitable for such records as were desired is a standard Lombard pattern with a very light rubber membrane 18 mm. in diameter and a very light bamboo lever with a paper tracing point, the whole lever arm being 9 cm. long. It is pivoted about 2.5 mm. from the axis. The force acts during a very short time and doubtless the momentum developed in the tracing lever serves to carry it beyond the point where the force ceases to

\*Submitted to the National Research Council. From the Department of Anatomy, University of Michigan Medical School, Ann Arbor, Mich.

Two previous reports have been made; the first was published in this JOURNAL, September, 1917, the second, January, 1918.

act, thus drawing out the membrane of the tambour. Instead of being the drawback which this would be with many kinds of tambour work, it serves here to indicate the very factor desired; namely, the force of the detonation wave that strikes the membrane of the tambour (in actual use of the preventives it would be the tympanic membrane). As expected, the writing lever rebounded a few times after the first movement, tracing a curve with very sharp apices and entirely without secondary components. It is only the distance of the first apex above the level of the base line that is of value here, and this is the distance referred to in the tabulated results. This, of course, indicates the compression phase of the wave; the method is not suited to obtaining the rarefaction component also, because of the momentum of the system.

Detonation waves of three degrees of force were used; they were generated by the firing of revolvers of 22, 38 and 44 calibers. The cartridges were blanks in all cases and were Peters' 22 shorts, U.M.C. 38 long Colt's, and U.M.C. 44 Colt's. The guns were fastened in a constant position by means of clamps attached to a plank fastened to the floor in an upright position. The detailed position varied slightly for the different guns, but was constant for the series of experiments with each gun. The 22 caliber revolver was fastened with the muzzle at a distance of 6 cm. from the orifice of the meatus of the rubber ear, with the gun pointing downward 25 degrees and toward the plane of the wall in which the ear was fastened at an angle of 12 degrees from parallel. The distance from the muzzle to the point where the line of fire intersects the line projected perpendicular to the wall at the meatus is 5 cm. It will be seen from the above that the shooting was as close as could be done without actually entering the ear with the flash of burning powder. The detail measurements for the positions of the larger guns were slightly different, but the essential arrangement was the same; all pointed slightly toward the plane of the wall with the ear at from 6 to 7 cm. from the muzzle and just out of line of the flash of powder.

As was stated in the animal experiments report, I fully realize that the detonations used are not of the initial intensity of those caused by the firing of artillery or by the explosion of shells or mines, but the distances used compensate to such a degree that with the animals severe injuries resulted when they were not protected; and in these physical tests the detonation force is constant except for cartridge variation; this permits a relative testing of the devices even though the absolute force of the detonations is less than that caused by larger explosions. Both methods of testing are well suited to using near large guns, and such tests could be readily performed at target practices or munitions proving grounds if proper authority and a few laboratory facilities were provided. Such tests would be an interesting check on these.

The guns were fired from the tambour room by means of a cord acting over pulleys, so that I was able to be with the recording apparatus and be sure that it was operating properly at all times. The kymograph speed used was such as to spread the apices of the after-vibrations sufficiently to avoid any confusion with the primary movement.

## PREVENTIVES TESTED

The names of the preventives tested are listed in Table I, descriptions of the eight which were also used in the animal experiments have been given in that report.\* Besides these eight, cotton soaked with water and the Elliott "Swimmer" were tested. This latter is a device manufactured by the same firm that makes the Elliott Perfect Ear Protector and is sold by them for use by swimmers to keep water out of the ear and to prevent unpleasant sensations when diving. It is very similar to the ear protector and consists of a celluloid core with three spaced discs of rubber; two light ones enter the meatus, and the third heavy one rests on the orifice. The celluloid center does not have a hole in it as does that of the protector.

## RESULTS

Three or four shots were fired with each device in each series except that with the 44, where only two shots were fired in some cases. The averages of the heights of the first apices of the resulting curves are given in Table I. In Table II are presented in graphic form the results with the 44 caliber revolver, with which all the preventives permitted enough of the force of the waves to pass to give measurable tracings. All measurements were made with a Bunne curve analyzer with a vernier permitting readings accurate to 0.05 mm. Because of the simple nature of the curves themselves, it is not necessary to figure them in this report, since it is only the height of the first apex that has value in these tests.

TABLE I

AVERAGE HEIGHT IN MILLIMETERS OF THE CURVES RESULTING FROM THE SEVERAL SHOTS WITH EACH DEVICE\*

PREVENTIVE DEVICE IN MEATUS OF RUBBER EAR	22 CALIBER REVOLVER	38 CALIBER REVOLVER	44 CALIBER REVOLVER
Nothing (ear open)	25.76	50.77	61.67
Dry cotton (packed firmly)	18.58	33.27	56.50
Elliott Perfect Ear Protector	17.76	31.22	54.65
Elliott "Swimmer"	12.40	29.15	48.77
Wilson-Michelson instrument	24.05	14.86	13.42
Water-soaked cotton	....	3.84	1.17
Wax Cone of Italian Navy type	0.41	5.02	0.50
Mallock-Armstrong Ear Defender	0.18	0.27	1.02
Glycerin-soaked cotton	Trace	0.38	0.35
Vaseline-soaked cotton	Trace	0.07	0.30
Scientific Ear Drum Protector "Tommy"	0.13	0.27	0.27

\*For the ammunition used and the arrangement of the guns and apparatus see the text matter.

## DISCUSSION OF THE RESULTS

In considering these results it should be kept in mind that probably the movements of the tambour are not a direct proportionate measure of the force; but that of two movements, the greater indicates relatively greater force than that implied by the ratio of the movements because of the increasing tension of the membrane of the tambour. No attempt has been made to calibrate the forces needed to cause the movements given, because the suddenness with which

\*Jour. Lab. and Clin. Med., Jan., 1918, iii, p. 226.



the detonation wave acts doubtless affects the movements as compared to that produced by a constant air pressure such as is used in calibrating. For the above reasons the results are fully recognized by me as indicating only the relative order of efficiency; not as being absolute.

The first thing noticed in studying the results is the division of the preventives into two distinct groups on the basis of the efficiency in stopping the force of the detonation waves. The ones that permitted the detonations to cause large movements of the tambour are the same ones that permitted the greatest injuries to the middle ears of animals. To these latter is added here the Elliott "Swimmer," which was not tested on animals. It is better than

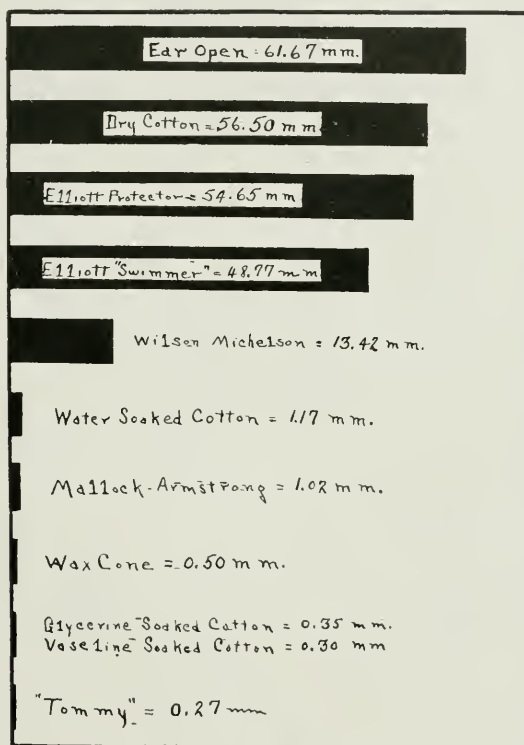


Chart II.—A graphic presentation of the results with the 44 caliber revolver.

the Elliott protector, but would certainly permit positive damage to the ears under conditions similar to those used with the animals.

On the other hand, the Scientific Ear Drum Protector "Tommy," which the middle ear conditions indicated as the best of the devices tested, has continued to be the device of choice. The Mallock-Armstrong Ear Defender is not quite so effective with these tests, but continues to be in the same group. To the objections for military use which I have previously urged against this device I have another to add now. The diaphragm of one of the pair of devices which I have has split and accordingly the device is greatly decreased in efficiency. With the damaged device in the meatus of the rubber ear, three shots from the 22 revolver gave tracings averaging 13.97 mm. While such splits

might not occur very often, yet when one did occur, the chances are entirely against the user knowing of it until he is injured because the diaphragm is partially concealed by the wire gauze. The metal diaphragm of the original 1915 patent is to be preferred so far as this score is concerned.

The glycerin- and vaseline-soaked cotton plugs have given better results here than in the animal tests; they are the only preventives that have not remained in the same relative positions in the order of efficiency as tested by the two methods. When opportunity arises, I hope to repeat the animal experiments with these soaked cotton plugs and see whether they duplicate the former results. As I remarked in the animal report, the universal availability of vaseline and cotton renders the degree of protection afforded by the mixture of importance to those to whom good mechanical devices are not available. These soaked cotton plugs cut down the hearing of ordinary sounds more than any of the other devices; this is, of course, an important objection to their military use.

The discrepancy in the results with the wax cones is probably due to the temperature. When the 44 was used, the room was very cool, while when the other series were run, the room was much warmer. I believe this is the correct explanation of the discrepancy. If so, it indicates a firmer wax for use in the human meatus, where a temperature warmer than that in the room would be obtained. Gianturco recognized temperature effects and suggested different mixtures for summer and winter use.

The discrepancy in the water-soaked cotton may be due to two factors; either a difference in the thoroughness with which the cotton was wet on the two occasions, which would be a factor impossible to control in actual use, or a more rapid evaporation may have occurred on the warmer evening. This latter is indicated by the fact that with the 38 the four shots fired gave successively increasing movements of the tracer, the actual measurements being 1.4, 3.35, 5.10, and 5.50 mm. Water-soaked cotton in the human meatus would so soon become dry cotton that it can not be recommended even though it is a fair protection when thoroughly wet. However, in an emergency it would certainly be advisable to wet cotton instead of using it dry and it would be well to rewet it frequently if opportunity could be found.

The behavior of the Wilson-Michelson device is interesting for it is both relatively and absolutely more effective with the heavier waves. It seems paradoxical at first, but when the structure of the device is considered, it is seen to be the logical result. The inertia and friction of the valve are more quickly overcome by the heavier waves and it is carried shut enough sooner to render the total energy that passes the valve less in the case of the more intense waves than with the slower action with the smaller waves. However, in all cases it acts too slowly to protect well; it will be recalled that with the animals it uniformly permitted severe injury to the middle ear parts with a .45 caliber pistol firing ball cartridges, with which the detonation was probably stronger than with the 44 blanks.

I would call attention to the fact that the animal experiments are the ones which indicate the devices that permit an injurious amount of detonations to pass; these physical experiments are of value in helping to place the preventives in their relative order of efficiency. The cochleæ of the animals, as ex-

plained in the middle ear report, will furnish a further check on the devices.

The fact that both methods of testing give concordant results indicating a relatively poor protection by dry cotton, by the Elliott Perfect Ear Protector, and by the Wilson-Michelson instrument in comparison with the protection afforded by other preventives leads me to express the wish that the proper military authorities will recognize the facts and take action to stop the use of the poor protectors by the troops and will furnish them with the better ones.

#### ANNOUNCEMENT

The method of testing here reported will enable me to make a determination in a few days after receiving it of the efficiency of any new device that may be submitted. Such a testing would place the new device in its relative order among the others and thus give a very fair indication of its effectiveness in protecting the ear itself. The more time-consuming animal testing could then be used as a check on the physical tests.

#### ACKNOWLEDGMENTS

I wish to here acknowledge my great indebtedness and to express my thanks to Dr. W. P. Lombard for the way in which he has placed the facilities of the physiologic laboratory at my disposal for these tests, and for the many helpful suggestions he has made in the course of the work. My thanks are also extended to Dr. Cope, Instructor in Physiology, for suggestions and aid with apparatus, and to Mr. P. M. Ireland and Mr. K. C. Kerwell, medical students, and to Sheriff Lindenschmidt for the use of revolvers.

# A CONTRIBUTION TO THE PHYSIOLOGY AND PHARMACOLOGY OF CHELONIAN LUNGS\*

By D. E. JACKSON, PH.D., M.D., AND MORT D. PELZ, ST. LOUIS, MO.

THE peculiar anatomic development of chelonians render these forms especially well suited for the study of certain phenomena relating to the innervation, physiology, and pharmacology of the lungs. In the year 1863 Mitchell and Morehouse<sup>1</sup> published a paper describing the musculature and mechanical actions by which the respiratory movements are carried out in these animals. In the present investigations we have confined ourselves to a study of the nature and course of the nerves controlling the intrinsic muscles of the lung substance, and to a few preliminary observations on the character of the responses of the lungs to certain drugs. Ordinary fresh-water American turtles have been used.

The method employed in these experiments is new and was devised in this laboratory about two years ago.<sup>2</sup> In the year 1909 Prevost and Saloz,<sup>3</sup> working in Geneva, studied certain features of the nervous control and drug reactions of the lungs in the tortoise. The method used by them consisted in making a round trephine opening through the carapace over the anterolateral region and then placing tightly in the trephine opening a cork through which a glass tube connected with a recording tambour was passed. The lungs were then rhythmically inflated by an artificial respiration machine, the air being conducted into the lungs by a cannula tied in the bronchus corresponding to the lung under observation. In this manner a number of observations were made by these authors. Most of these we have corroborated, but in a few instances we have failed to obtain such results as were reported by them. In addition to their work, which, so far as the innervation was concerned, covered only the vagal constrictor fibers, we have found direct evidence of the existence of a definite set of sympathetic dilator fibers, and we have also obtained results indicating that certain constrictor fibers are to be found running in the sympathetic nerves.

The simplicity of this work, together with the small amount of apparatus needed and the definiteness of the results, render these experiments peculiarly well suited for ordinary class exercises. There are a few striking differences in certain drug reactions between mammalian lungs and those of the turtle, but from a teaching standpoint these may be very valuable by way of comparison.

Reference to Fig. 1 will show the chief features of the method used by us. The turtle is thoroughly pithed, both brain and cord. The plastron and viscera (except the heart and lungs) are then removed and the skeletal musculature is dissected out of the carapace as thoroughly as possible, care being taken not to puncture the lungs which in the meantime may well be partially inflated by means of a cannula tied in the exposed trachea. The vagosympathetic nerve trunks are isolated in the neck and the bronchus to the lung *which is not to be studied* is closed off with a bulldog clamp. The other lung is connected by tub-

\*From the Department of Pharmacology of Washington University Medical School, St. Louis, Mo.

A preliminary report of this work was given before the Federation of American Societies for Experimental Biology at Minneapolis on Dec. 27, 1917.



ing to a delicate tambour having a bowl about one and one-half to two inches in diameter. A high magnification is used and the lung is blown up to about one-half its capacity. This puts the air tension in the lung and in the tambour on a balance and a contraction or relaxation in the lung will show an immediate rise or fall of the writing point of the tambour.

Fig. 2 shows a diagram of the lung innervation in the majority of turtles studied by us, but there are considerable variations in different specimens of the same species, while in a few varieties (especially snapping turtles) we have not been able to recognize the sympathetic dilator fibers. It is extremely probable,

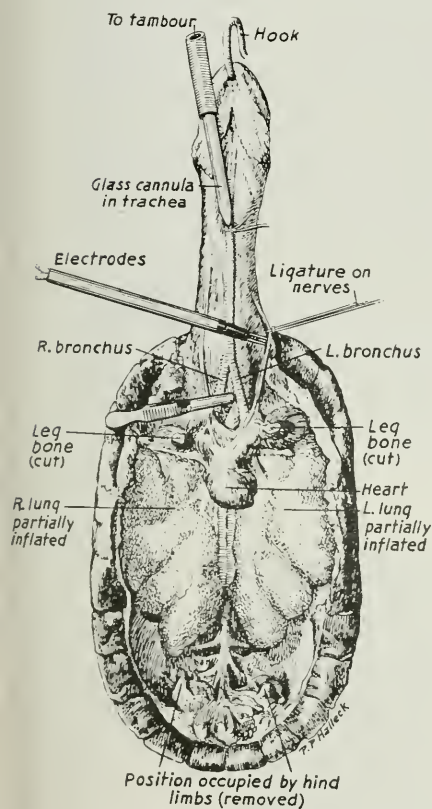


Fig. 1.

Fig. 1.—Dissection showing method of removing the plastron, viscera (except the heart and lungs and large blood vessels), and skeletal muscles. In this way each lung may be observed directly while the records are being made.

Fig. 2.—Diagrammatic representation of the innervation of the lungs in the turtle. Right lung, ventral view. For discussion see text.

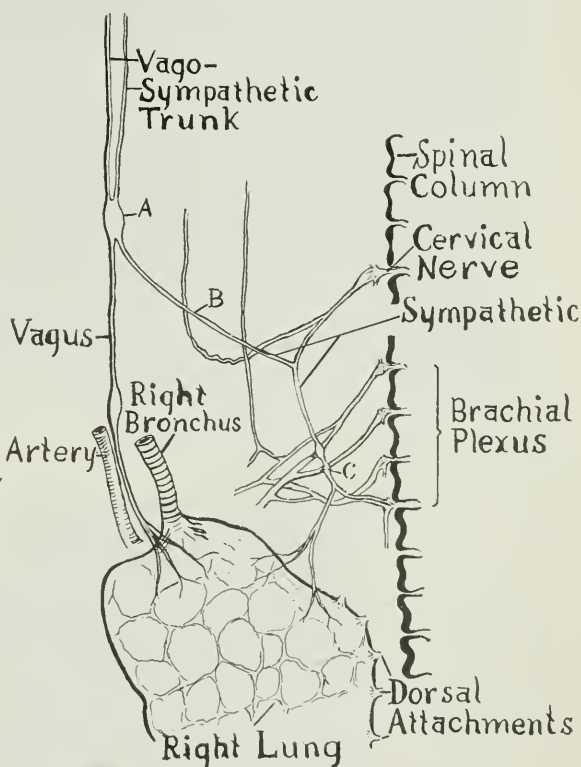


Fig. 2.

however, that a more careful search would reveal the presence of these fibers in this species the same as in others. Apparently seasonal variations are also present in the action of these nerves. Probably these reasons are sufficient to explain our failure in some experiments to satisfactorily demonstrate either constriction or dilation of the lungs following electrical stimulation of the nerves.

At the ganglion marked *A* in Fig. 2 a very small sympathetic nerve bundle labeled *B* joins the vagosympathetic trunk. If the nerve *B* be stimulated with a *very weak tetanizing current*, a dilatation of the corresponding lung usually

follows, but if a strong induced current is used a contraction of the lung is much more frequently produced, and this contraction sometimes follows more vigorously and promptly than even that which is produced by stimulation of the vagus trunk itself. These results we believe justify the conclusion that in some turtles (if not in all) the sympathetic nerves carry both dilator and constrictor fibers for the lungs. These fibers are very small and difficult to isolate without injury,

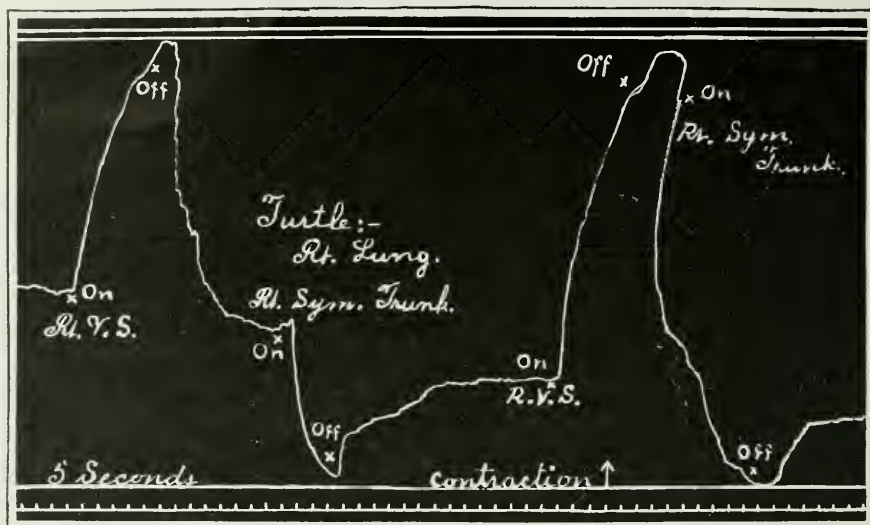


Fig. 3.—Tracing showing the contraction of the right lung following vagus stimulation, and relaxation produced by stimulation of the right sympathetic chain (at C in Fig. 2).

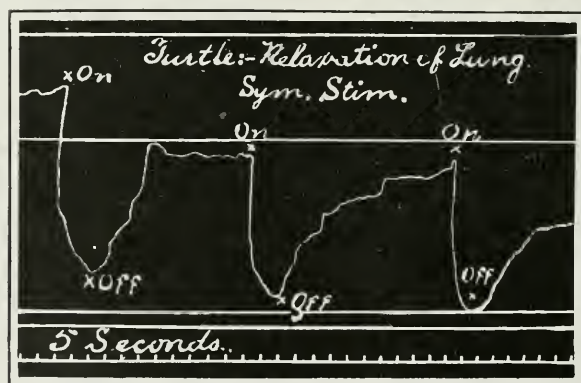


Fig. 4.—Tracing showing dilatation of the lung produced by sympathetic stimulation.

and this sometimes makes the experiment difficult or ends in failure. The dissection should be made by lifting the vagus nerve upward and outward from the base of the neck while the sympathetic branch marked B is followed down toward the main sympathetic chain at C.

If the main sympathetic chain in the region of the ganglion marked C, or on the ganglion just above this, be stimulated with a *weak tetanizing current*, a dilatation of the corresponding lung will be produced immediately. The dilata-

tion is most marked in the upper portion, that is, in the region around the entrance of the bronchus and in that part of the lung which lies lateral to this. Probably this is due to the better developed musculature in this region, for the posterior or caudal part of the lungs is much thinner and more membranous in its structure.

Figs. 3 and 4 show typical results obtained by stimulation of the vagus and of the sympathetic fibers. Stimulation of the vagus trunk causes stopping of the heart and lung contraction, but sympathetic stimulation, preferably always with a weak current, causes pulmonary relaxation and either no effect on the heart, or possibly occasionally some acceleration of the beat.

Regarding the response of the turtle lung to drugs, it may be said that nicotine, pituitrin, lobelin and veratrine cause marked contraction, usually of a prolonged nature. These drugs we have generally applied by injection with a small hypodermic syringe directly into the beating ventricle. The solutions are soon carried around to the lungs. None of the above drugs produce either a marked or prolonged contraction of the bronchioles in the dog. Codeine, pilocarpine, etc., act in the turtle apparently in practically the same manner as in the mammal.

We have repeatedly tried to get satisfactory results by the use of atropine and adrenalin. After atropine we have sometimes seen what undoubtedly appeared to be a direct muscular contraction, but we have not been able to follow this result up closely and it may possibly have been in each case the result of some technical error, but we hardly believe this to be the true explanation. With adrenalin we have not so far obtained a typical dilation of the lung as would occur in the case of the mammalian bronchioles. Possibly a constant perfusion of the lungs with a suitable solution to which adrenalin was added at the proper time might show a pulmonary dilatation, but the results are open to doubt at present.

#### BIBLIOGRAPHY

- <sup>1</sup>Mitchell, S. Weir, and Morehouse, G. R.: *Anatomy and Physiology of Respiration in the Chelonia*, Smithsonian Contributions, 1863, xiii.
- <sup>2</sup>Jackson, D. E.: *Experimental Pharmacology*, 1917. C. V. Mosby Co., St. Louis, 1917, p. 260.
- <sup>3</sup>Prevost, J. L., and Saloz, J.: *Arch. internat. de physiol.*, 1909, viii, 327.

# BACTERIOLOGIC FINDINGS IN OZENA—SECOND REPORT\*

BY HERBERT C. WARD AND DONALD C. BEAVER, DETROIT, MICH.

THE etiology of chronic catarrhal infection demands more serious attention by the bacteriologist than it has yet been given. Available records repeatedly indicate that invasion of the upper respiratory tract may take place very early in life, children of three and four years becoming subject to pronounced forms of rhinitis from which they never recover. Our clinics register daily the ozenic and atrophic, the tuberculous or syphilitic adult of forty years. In fact there is no lack of fresh material or willing patients for the needs of serious investigation. Both are abundant from various sources and climates and of all ages.

In tabulating the cases coming to this laboratory for observation we have found it convenient to make the following distinctions. The diagnosis of atrophic rhinitis is indicative of atrophy, chronicity of discharge, crustal formation, and impairment of free nasal respiration. The term ozena refers to the obvious symptom of malodor. Inasmuch as ozena is closely associated with atrophic rhinitis and frequently presents all the symptoms of the same, it is here considered as a frequent symptom of atrophic rhinitis, and not ozena, a clinical entity. By definition, therefore, causative factors of the atrophic and also of the ozenic stage may not be identical. Investigation of this constitutes the objective of our study on some seventy cases. One hundred and fifty bacteriologic analyses were conducted as completely as the time and facilities were convertible for this work. The findings agree with those of the first paper† and have yielded very suggestive records.

The most comprehensive report published in this country is that of Horn of San Francisco. He suggests that ozena is not a clinical entity, meaning that more than one biologic agent may bring this condition about. After reviewing previous work and supplementing the same with a series of experimental vac-

TABLE I

<i>B. mucosus capsulatus</i> (Abel's bacillus)	appeared in	42	cases, or	84%
<i>B. diphtheroids</i>	"	37	"	74%
<i>M. staphylococci</i>	"	37	"	74%
<i>B. Perez Type II</i> (atypical)	"	34	"	68%
<i>M. streptococci</i>	"	31	"	62%
<i>B. proteus</i>	"	14	"	28%
<i>B. Perez Type I</i> (typical)	"	11	"	22%
<i>B. influenza</i>	"	9	"	18%
<i>M. pneumococci</i>	"	8	"	16%
<i>M. catarrhalis</i>	"	6	"	12%
<i>B. coli</i> and <i>pyocyaneus</i>	" (each)	3	"	6%

cines, he proceeds to show that bacteriologically all his cases fall into two groups, one designated as the "Friedlander" and the second as the "Perez" group. Lit-

\*From the Research Laboratory of Parke, Davis & Company, Detroit, Mich.

†Bacteriologic Findings in Ozena, First Report, Jour. Infect. Dis., Aug., 1916, xix, 153.



erature of the subject reveals similar conclusions from the earliest of the investigations and a previous report from this laboratory records identical findings.

Bacteriologic studies have aimed to include the most important groups of organisms so far as routine analyses and methods would permit. Table I includes the findings obtained from fifty cases diagnosed as ozena (atrophic rhinitis fetid). The groups are arranged in the order of their predominance in the fifty cases.

These cases are representative of conditions found in this country, having been taken from a wide radius as follows: Michigan, 25 cases; Louisiana, 5; Illinois, 2; Minnesota, 3; Ohio, 7; California, 4; and Kentucky, 4. Inasmuch as the material had to be shipped a long distance before being subjected to bacteriologic examination, the thoroughness of the analyses was limited to but one trial in the majority of the cases.

Reviewing Table I, it is evident that the preponderance of *B. mucosus capsulatus* is most significant. An organism similar to this and known as the Morax-Axenfeld bacillus appeared only a few times and is not, therefore, considered

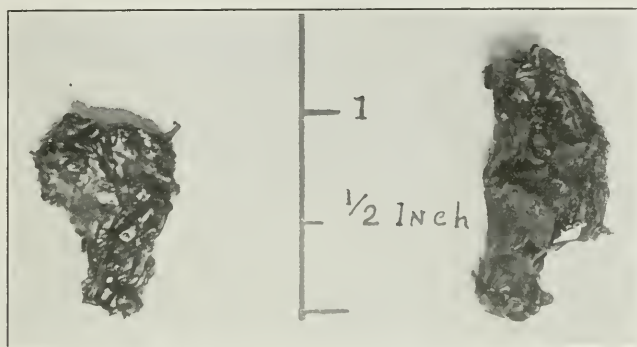


Fig. 1.—Fresh nasal casts formed in 48 hours from two girls, 10 and 12 years old, respectively, diagnosed as atrophic rhinitis cases. The records give histories of two and five years' duration.

as being important. It is easily confused with the *B. mucosus capsulatus* microscopically, but never culturally.

Diphtheroid bacilli have taken second place in the table. In two instances toxin-producing bacilli were abundantly present and the cases were classed as diphtheria carriers by the local board of health. The identification of such organisms from patients suffering from chronic catarrh is most suggestive for practical investigation bearing on the problem of diphtheria carriers. No work of this character has as yet been called to our attention.

The much discussed Perez group of bacilli has appeared in a higher percentage of cases than in the first census. The strains isolated do not conform, on careful study, to the reported type. Two groups are distinguished. Type I represents a class conforming to the standard strain. Type II represents atypical Perez strains, differing from the stock in their agglutination, fermentation, and motility tests. Neglecting the question of strain identity for the moment, the significant fact appears that some representative of the Perez group is found in forty-five cases out of the fifty. Of additional value is the discovery that those cases contain no Perez types, but in their place, bacilli set down as *B.*

*proteus vulgaris*. The Perez and the proteus groups are, therefore, responsible for the malodor of ozena cases.

The remaining groups of organisms appear at present to be of little or no significance.

Supplementary to the fifty ozena cases just reviewed, the following group,



Fig. 2.—Original nasal mucus field. *B. mucosus capsulatus* types as seen with the Gram stain.

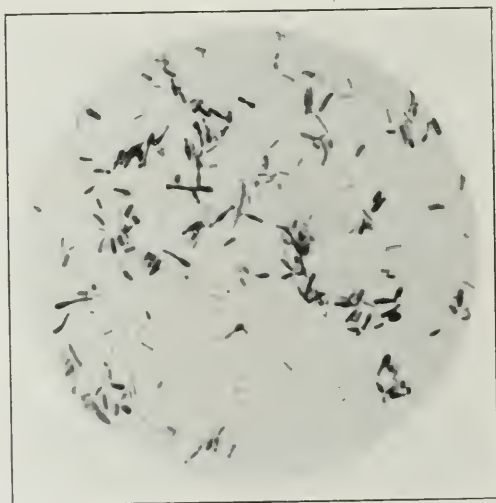


Fig. 3.—Original fields filled with the omnipresent representatives of the *B. diphtheroids*.

diagnosed as "atrophic rhinitis," and differing from the former only by the absence of odor, is tabulated. Material for bacteriologic study was received from: Michigan, 14 cases; Kansas, 1; Minnesota, 2; Illinois, 1; Ohio, 1; Indiana, 1; and Wisconsin, 1, making a total of 21.

TABLE II

B. mucosus capsulatus	appeared in 17 cases, or 81%
M. staphylococcus	" " 16 " " 76%
M. streptococcus	" " 14 " " 70%
B. diphtheroids	" " 12 " " 60%
B. influenza	" " 3 " " 15%
M. catarrhalis	" " 3 " " 15%
B. spore bearers	" " 2 " " 10%
B. pyocyaneus	" " 2 " " 10%
B. Morax-Axenfeld	" " 1 " " 5%
B. Perez	" " 1 " " 5%
B. proteus	" " 1 " " 5%

Two facts are strikingly evident; first, that the capsulatus group is the most abundant; and, second, that the Perez and the proteus representatives are the least abundant. In this connection it is to be noted that the patients when examined at the clinics showed no odor. The nasal discharges, when incubated, developed no odor (except in two cases) and yielded no cultures giving odors. The results suggest that the existence of a Perez-proteus infection can be recognized in the majority of cases by clinical examination alone, and in such instances bacteriologic examination is unnecessary.

A summary of our study shows that the group of organisms known as the B. mucosus capsulatus (Friedlander's bacillus, Abel's bacillus) is preponderant and may be pathognomonic in cases of chronic rhinitis. This group is also independent of the action of putrefactive bacteria. In cases in which the symptom of malodor exists, a different class of bacilli known as the Perez group is most abundant and all cases show either one or both of the Perez and proteus groups present as causative factors of this condition.

Cases of chronic catarrh may harbor pathogenic organisms of other species such as the diphtheria bacilli and contribute to the distribution of similar infections.

## A CASE OF SYMMETRICAL PERIPHERAL GANGRENE\*

BY CHARLES E. KIELY, M.D., CINCINNATI, OHIO.

PATIENT No. B. 4534, age 39 years admitted to the First Neurologic Service of the Cincinnati General Hospital on June 19, 1917. His chief complaint was "nervousness" and extreme tenderness with marked cyanosis of the hands. He was under the effect of alcohol.

The history developed the following facts; he had had the usual infections of childhood; at fourteen contracted a hard chancre which was treated by internal medication only and followed by an inconspicuous secondary macular eruption on the legs; admitted gonorrheal infection twice; had pleuropneumonia at 37; had been drinking to excess for five or six years.

The history of the present complaint was that for five or six weeks prior to admission he had been more than ordinarily excessive, averaging about one quart of whiskey daily. About two weeks before admission he had taken five migraine tablets for headache in about forty-eight hours. Two days before admission both hands began to turn blue and became exquisitely tender to touch. He was an electrical engineer by profession and could recall using no reagents which might have produced a local condition.

Physical examination revealed a fairly nourished and developed conscious adult white male whose most conspicuous objective symptom was a marked cyanosis of the more distal portion of the hands and of the fingers, as well as of the corresponding portions of the feet and toes. The lips were only slightly ashy, and the rest of the body surface of a grayish tinge.

In the cyanotic areas the hands and feet were very cold, and at this first examination generally more tender to light than heavy pressure, though even then deep pressure on the thenar muscles was extremely painful. Subjective pain was constant and severe, and he could only find relief by constant application of ice bags and in addition morphine became a constant necessity soon after admission. At all times both pulses could be felt at the wrist and ankle, nor did the vessels on palpation give the impression of sclerosis. Blood pressure was 134 systolic and 100 diastolic. Further notes on the physical condition are: purulent nasal discharge; tongue heavily coated; teeth and gums neglected with marked pyorrhea and very offensive breath; pharynx congested; chest symmetrical but narrow with whistling rales heard over both lungs posteriorly; heart action regular, not enlarged, sounds easily audible and without murmurs; abdomen negative.

*Mental Examination.*—Patient was conscious and cooperated well. His attention was good and orientation for time, place, and personality correct. Emotional attitude was in proportion to his situation. Cerebration commensurate with his station and opportunities.

*Neurologic Examination.*—Smell, taste, sight and hearing normal. The pupils reacted rather slowly to light, by both direct and indirect illumination,

\*From the Pathological Institute of the Cincinnati General Hospital.



but well on accommodation. There was no squint, ptosis or nystagmus, ocular movements were free in all directions.

The jaw did not deviate from the median line on opening the mouth. Both masseters contracted equally and with normal power on voluntary effort. The corneal reflex and facial sensation were undisturbed. All facial folds were normal, and the periorbital and perioral muscles contracted normally in voluntary and emotional response. In articulating there was some slight indefinable thickness of speech, but deglutition was normal. There was nothing to suggest abnormality of the muscle supplied by the eleventh nerve, and the pulse rate was not subnormal. The tongue protruded in the median line on voluntary effort, showed some tremor but no atrophy. The somatic muscles were of normal tone and development, and movements of the limbs were of proper range, power, and coordination.

*Sensory System.*—Heat, cold, pain, touch, and position sense were normal, with the exception noted above for the thenar muscles. The skin reflexes were absent. The tendon reflexes showed a slight symmetrical exaggeration, there was no clonus, Babinski or Chaddock.

The urine was cloudy, red, with specific gravity of 1,012, neutral and gave no sugar reaction, but a marked albumin ring. Microscopically it contained blood and hyaline casts. Red count, 3,460,000; white count, 7,600, hemoglobin, 87 per cent (Sahli): A spectroscopic examination of the blood showed hemoglobin bands, but none of methemoglobin.

The patient was seen on the twenty-fourth of June by Prof. R. S. Morris, who gave the following opinion:

"No petechie. Heart not enlarged. Sounds strong and clear. Lungs clear on percussion. Vesicular breathing throughout. An occasional squeaking rale over both lungs. In upper left interscapular space, a few crepitant rales. Radial pulse regular, good volume, vessel walls feel about normal.

"*Abdomen.*—No localized rigidity, no masses. Tympany in flanks. Spleen not enlarged by palpation or percussion.

"Gangrene of hands and beginning gangrene of feet suggest a localized endarteritis. An arterial thrombus would seem to be a possibility, especially in view of the blood in urine and stools, but the absence of petechiæ, and the symmetrical involvement are against it."

In the next two days the patient's general condition became worse and the duskiess of the skin became more general and the fingers as far down as the middle phalanges developed a typical gangrene of the dry type. A line of demarcation forming across the middle of the hand. The respiration became of the Cheyne-Stokes variety and on June 26 the patient died.

*Clinical Diagnosis.*—Acute and chronic alcoholism; spontaneous symmetrical gangrene.

#### AUTOPSY (J. S.)

The body was that of a well-developed, well-nourished adult white male about 40 years of age. Postmortem rigidity was present and well marked. Postmortem lividity was brilliant over the back and buttocks. The scalp and skin over the face and neck, ears and scrotum were markedly cyanotic. The

lips and gums were also cyanotic. There were numerous other large areas of cyanosis over the arms and legs. The toes were not cyanotic, but the great toes showed areas of hyperemia which were of a dark red color and tended toward a blue color along the edges. There was no evidence of any shriveling or hardening of any of the toes.

The fingers and thumbs of the hands were markedly cyanotic, shriveled, rigid, and flexed. The nail-beds were very cyanotic. The ends of the fingers were dark blue (almost black), hard and dry as far back as the proximal end of the middle phalanges. Here there was a distinct line of demarcation beyond which the color was of a lighter blue and the skin was softer and the joints were not so rigid. This area extended backward over the fingers and hands to about a half inch from the proximal ends of the metacarpal bones. This area extended farther upon the sides of the hand, as far up as the lower end of the ulna on the ulnar side and as far up as the lower end of the radius on the radial side. Here there was a second distinct line of demarcation beyond which the skin was somewhat cyanotic but not as deeply so as distally to this line. The flexed fingers were so rigid that they could not be straightened out. The blood vessels, especially the veins, in these cyanotic areas contained blood clots which had completely filled the vessels.

The cranium and spinal canal were opened and the brain and cord were removed. There was some edema of the brain and a moderate degree of congestion of the vessels of the brain. No lesion in the cord or brain could be noticed by the external examination, and gross section (C.E.K.) showed no abnormalities.

Upon opening the chest, the lungs partially collapsed. The left lung was free from adhesions and crepitated in its lower lobe, but had a shotty feel throughout its upper lobe. Upon section, the cut surface presented an edematous and slightly congested appearance and many miliary tuberculous nodules were visible in all stages in the upper lobe. The right lung was bound down at its apex and to the diaphragm by old fibrous adhesions. The cavities were easily seen in the apex. The largest of these cavities measured about 2 cm. in diameter. This lung was also crepitant in its middle lobe and felt shotty in the other lobes. Upon section, many tuberculous nodules were visible and cavities had been found in the upper lobe only. These cavities had thin walls. The middle lobe was only slightly congested. Many of the peribronchial glands were enlarged, and upon section were seen to have undergone calcareous changes. The pleural cavities each contained about 150 c.c. of free light amber-colored fluid.

The heart was slightly enlarged. The right heart was normal and contained a large postmortem clot. The pulmonary artery and valve were normal. The left auricle was normal, but there was a marked thickening of the mitral valve. The musculature of the heart was thicker than normal and fairly firm. It was pale in appearance. The aortic valve was normal. The aorta showed many fatty and calcareous changes throughout its entire course, but more especially in the ascending and transverse arch.

The abdomen was opened and the intestines were distended with gas and the vessels were deeply injected. The appendix was *in situ* and normal in

appearance. The stomach epiploic vessels were also deeply injected. The stomach was of normal size and shape, but the mucous membrane showed a marked thickening and very deeply injected, streaked, red and yellow appearance. The pylorus was normal and easily admitted the tip of the index finger.

The liver was of normal size, smooth on the surface and of a dark congested appearance. The gall bladder and ducts were apparently normal.

The spleen was about normal size of a dark blue color and very soft. Upon section the follicles were easily visible and the surface had a congested appearance.

The right kidney, after some difficulty, was found in the normal position. The kidney was about the size of a kidney of a newborn infant. It presented a normal appearance externally, except for its size. Upon section, the cut edges everted and the cortex was well marked off from the medulla. The left kidney was somewhat larger than normal, but not so large as would have been expected with such an infantile right kidney. There was a dark purple swollen and softened area on the inner side of the kidney towards the lower pole, which only extended into the cortex of the kidney and appeared to be necrotic. Upon section, the cut edges everted, the cortex was thicker than normal, and not well marked off from the medulla. Throughout the kidney on the surface there were numerous whitish miliary areas resembling tuberculous areas or abscesses. These areas contained whitish soft caseous-like material.

The bladder was slightly distended and contained about 200 c.c. of clear yellow urine. The ureters and adrenals were apparently normal.

The posterior tibial artery was dissected from the right leg and foot. The radial and ulnar arteries were dissected from the right arm and hand. The middle finger of the right hand was amputated at the middle of the proximal phalanx.

#### DISCUSSION

The obvious interest of this case is the differentiation from the Raynaud syndrome. A gangrene with arteriosclerosis in an admitted syphilitic hardly comes under that diagnosis. A review of the rather voluminous literature of Raynaud's disease leads one to believe that the diagnosis is often made with scant logic. Reading the actual translation of Raynaud's original thesis and his "Newer Researches" one accepts the picture of a symmetrical peripheral gangrene due to a spasm of the vessels as the entity which the author wishes to differentiate. That this can not be logically done unless vascular disease is ruled out is obvious. Yet one is unpleasantly struck with the inadequate amount of pathologic certification of his twenty-two cases. Only six came to autopsy and the nature of the report in these is far from satisfactory. For instance, in Case 7, "The autopsy revealed nothing special." In Case 9, "Careful injection and dissection of the arteries was negative." This is the only case which Raynaud examined microscopically in person. In Case 17, "The arteries were adherent to the bone and closed at the line of demarcation." In Case 18, "The mitral was sclerotic and there was narrowing of the entire arterial system." In Case 19, "Both auriculoventricular orifices were narrowed by

sclerosis." In Case 22, "Both femoral arteries were remarkably shrunk and were not patulous on division with the knife."

The trump card for the vascular spasm theory in this syndrome is the observation of contraction and expansion of the retinal vessels in definite time relation with the peripheral symptoms. It is regrettable to discover that Raynaud observed this phenomenon definitely in but one of his twenty-two cases.

Clashing further with his own definition, he has included one case in which the gangrene alternated between the sides of the body and two in which it was unvaryingly unilateral.

Coming down to recent writers, one finds equal illogicality. Montgomery and Culver<sup>1</sup> report a case involving the left hand only with a relapse from "psychic shock." During the course of the disease there was a difference of blood pressure of 10 millimeters which gradually abated as a cure ensued. Differences of 10 millimeters are rather unconvincing.

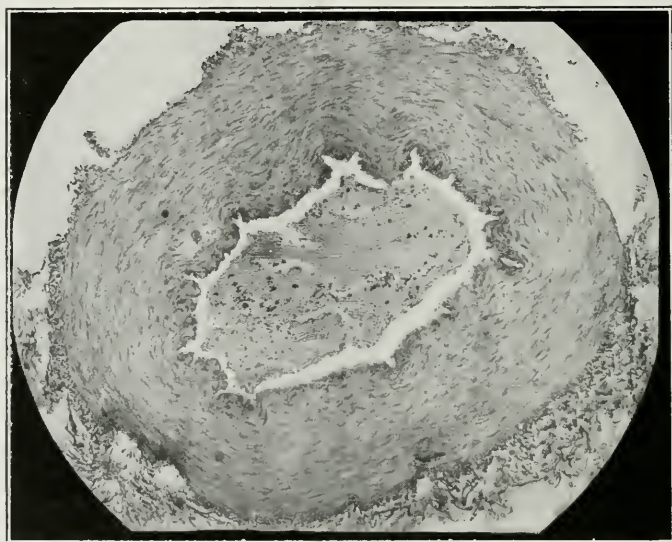


Fig. 1.

Osborne<sup>2</sup> reports a case of intermittent attacks of mental disturbance and mumbling in a known syphilitic who improved under salvarsan, and without any other evidence of vascular disease diagnoses "cerebral Raynaud."

In contrast there are many more carefully studied cases. Lisser<sup>3</sup> reports a case in a negro child with gonorrheal arthritis and a positive Wassermann reaction on the blood.

Glazer<sup>4</sup> reports a case in a child with hereditary syphilis which improved under mercury.

Lyle and Griewie<sup>5</sup> report a case in which autopsy revealed vascular sclerosis with small round-cell infiltration.

Simmons<sup>6</sup> reports a case not clinically syphilitic in which, on postmortem, slight thickening of the arteries without endarteritis was found.

Hoynes<sup>7</sup> reports the case of a child who in the course of forty-six days had



scarlatina, measles, and pertussis, developed symmetrical gangrene and died of bronchopneumonia. On autopsy streptococcus viridens and streptococcus hemolyticus were recovered from a femoral thrombus.

Buerger,<sup>8</sup> arguing for thromboangiitis obliterans as the cause of symmetrical peripheral gangrene, reports such a macroscopic diagnosis on vessels post-mortem and after amputation. One case examined microscopically showed "typical lesions of thromboangiitis obliterans."

Clinically the case here reported personally would pass well as a Raynaud's syndrome. The palpable portions of the radial were soft, there was hemoglobinuria and the local picture was perfect. But the postmortem examination places it in another group. Macroscopically higher portions of the brachial arteries were sclerotic, and microscopically this is excellently brought out in Fig. 1. The sclerosis is diffuse and small round-cell infiltration is lacking so that syphilitic sclerosis can not be absolutely predicted.

Undoubtedly the diagnosis of Raynaud's disease as made lacks logical substantiation, and the case above is offered in behalf of differentiation.

#### BIBLIOGRAPHY

A comprehensive bibliography is not offered, being too cumbersome and unlikely to improve on that compiled by H. Norman.<sup>9</sup> Cases quoted in the text above are noted below for reference.

<sup>1</sup>Montgomery, D. W., and Culver, G. D.: An Instance of Asymmetrical Raynaud's Disease, Jour. Cutan. Dis., 1915, xxxiii, 119-125.

<sup>2</sup>Osborne, O. T.: Raynaud's Syndrome: Raynaud's Disease, Am. Jour. Med. Sc., 1815, cl, 157-169.

<sup>3</sup>Lisser, H.: Syphilis and Raynaud's Disease, Arch. Int. Med., 1915, xvi, 509-516.

<sup>4</sup>Glazer, F.: Syphilis Hæmorrhagica Hereditaria oder Säuglings-Raynaud?, Med. Klin., 1914, x, 1136-1139.

<sup>5</sup>Lyle, B. F., and Griewie, John E.: Philadelphia Med. Jour., 1901.

<sup>6</sup>Simmons: Raynaud oder Endarteritis obliterans oder Embolie?, Berl. klin. Wchnschr., 1914, li, 1672.

<sup>7</sup>Hoyne, A. L.: Raynaud's Disease; a Report of a Case of Symmetrical Gangrene of Unusual Severity, Jour. Am. Med. Assn., 1915, lxxv, 1725-1729.

<sup>8</sup>Buerger, L.: Concerning Vasomotor and Trophic Disturbances of the Upper Extremities; with Particular Reference to Thrombo-angiitis Obliterans, Am. Jour. Med. Sc., 1915, cxlix, 210-229.

<sup>9</sup>Norman, H. J.: The Cerebral Associations of Raynaud's Disease, Lancet, London, 1916, 1049, 52.

# STUDIES ON DIPHTHERIA TOXIN\*

BY LEWIS DAVIS, S.M., DETROIT, MICH.

## I. HYDROGEN-ION CONCENTRATION AND TOXICOGENICITY DETERMINATIONS WITH BACT. DIPHTHERIÆ

**HISTORICAL RESUME.**—Some of the earliest workers with Bact. diphtheriæ have noted that the reaction of the culture medium has a decided influence on growth and toxin production with this organism. Roux and Yersin,<sup>1</sup> who appear to have been the first to extensively investigate this question, recommend the use of slightly alkaline bouillon, since they found that an acid reaction did not permit of strong toxin formation. Spronck<sup>2</sup> attributed this acid production to the presence of a variable quantity of glycogen and glucose, fermentable by Bact. diphtheriæ, in the meat used for the bouillon. To overcome this factor, he proposed the employment of decomposed beef or veal in which the putrefaction had reduced the content of these sugars to a minimum.

Park and Williams<sup>3</sup> found that with the beef used by them, the inhibitory action of the muscle sugar was neutralized by adding sufficient alkali to the bouillon (about 7 c.c. of normal soda solution per liter). They state "that an excessive amount of either acid or alkali prevented the development of toxin" and that the "type of growth of the bacilli and the rapidity and extent of the production of toxin depended more on the reaction of the bouillon than upon any other single factor."

Essentially the same results have been found by Madsen.<sup>4</sup> This investigator concludes that in the same bouillon, diphtheria cultures can develop in one of two directions, acid or alkaline, depending upon the initial degree of alkalinity. Confirming the results of Spronck, and Park and Williams, acid cultures were found by him to possess no toxicity, while, in general, the alkaline cultures are toxic, although no definite relationship exists between the degree of alkalinity and the amount of toxin produced.

In order to eliminate any action of the muscle sugar, Theobald Smith<sup>5</sup> proposed a peptone bouillon in which the beef infusion, previously reduced in acidity, was submitted to a preliminary fermentation overnight with *B. coli*, then sterilized and filtered. This would break up all carbohydrates, and give at once an alkaline growth with a constant, strong toxin. Addition of dextrose to such bouillon in quantities not exceeding 0.2 per cent, was found not only to be uninjurious, but actually led to a maximum accumulation of toxin, presumably by utilizing the available peptone to the best advantage. This method has not met with general favor, although Hitchens<sup>6</sup> reports good results from its use with veal bouillon. Lubenau<sup>7</sup> confirms the findings of Smith that no acid is formed in carbohydrate-free, nutrient bouillon, and also states that, as a rule, diphtheria bacilli produce alkali in such bouillon independent of the initial reaction. On the other hand, he finds that diphtheria-like organisms produce no appreciable amount of alkali, but leave a carbohydrate-free bouillon unchanged. In ordinary bouillon, which would contain carbohydrates, both diphtheria and diph-

\*From the Research Laboratory of Parke, Davis & Co., Detroit, Mich.

theria-like organisms produce acid, regardless of the initial reaction of the bouillon.

Jacobsen,<sup>8</sup> in a critical review of the work of Madsen and of Lubenau, agrees that in sugar-free bouillon (Smith method) diphtheria cultures produce alkali at once. He also finds that, not only has dextrose an influence on the acid formation, but with a constant amount of glucose present in a bouillon, the amount of acid produced increases proportionately to the peptone content. Any culture of *Bact. diphtheriæ* in bouillon can, according to him, theoretically be considered as passing through the following stages: primary acid formation, reversal, alkali formation, secondary acid formation. This last stage which takes place only after a prolonged period of time (two to four months) has its origin in the neutralization products of the above mentioned reversal stage and is different from the primary acid formation which is attributed to the muscle sugar present.

A consideration of the work mentioned above, in the light of the modern conception of reaction based on hydrogen-ion concentration, brings up at once the limitations of results obtained by the usual titrametric methods. In fact, it is now generally conceded that there is a decided difference between the acid and alkaline values obtained by titration using litmus and phenolphthalein. Clark,<sup>9</sup> in an able discussion of the fallacy of titrating hot media, states, "It is needless to add that since titrations in the last analysis are based upon the attainment of a certain hydrogen-ion concentration as shown by the tint of an indicator, the titration of a medium at 90° to 100° C. furnishes data of no exact significance at ordinary incubation temperatures."

In connection with the development of a satisfactory bacteriologic peptone, I<sup>10</sup> have employed the production of a potent diphtheria toxin with a trial sample as one of the biologic tests. This required a study of the factors influencing growth and toxin formation in bouillon of constant composition and, as would be expected from the preceding resume, the hydrogen-ion concentration of the medium has been found to be of prime importance. The present investigation was undertaken to determine more accurately by means of the hydrogen electrode what reaction changes take place in the medium during the propagation of diphtheria toxin on a practical scale, and to note what relationship, if any, exists between toxicogenicity in a medium and hydrogen-ion concentration.

#### LABORATORY DATA

*Methods of Experimentation.*—Extensive experience has shown that plain bouillon gives uniformly satisfactory results in the production of toxin with *Bact. diphtheriæ*. Sugar media and carbohydrate-free broth (Smith method) have not proved of any advantage. In the study at hand, unless otherwise noted, the medium employed was plain bouillon, made up as given in the following:

To every liter of beef infusion prepared in the usual manner, 20 grams of peptone (Bacteriologic, Parke, Davis & Co.) and 5 grams of sodium chloride were added. Supplies of sterile beef infusion and of the other two ingredients from individual lots were retained in quantities sufficient for all of the experimentation so as to eliminate any variations of the bouillon components. The peptone and salt were first dissolved in the cold, then heated in a steamer for fifteen minutes to insure thorough solution.

The medium was now ready for preliminary adjustment to the desired hydrogen-ion concentrations which was done essentially as proposed by Clark and Lubs,<sup>11</sup> using the buffer solutions, standardized by the hydrogen electrode, and the indicators recommended by them. As will be later shown, the limiting concentration values for cultivation of the diphtheria bacillus are approximately  $C_H^+ = 1 \times 10^{-6}$  ( $P_H^+ = 6.0$ ) and  $C_H^+ = 1 \times 10^{-9}$  ( $P_H^+ = 9.0$ ), respectively, which reduces the standard buffer solutions necessary to the "acid potassium phosphate-sodium hydroxide" mixtures and the "boric acid-potassium chloride-sodium hydroxide" mixtures. "Brom cresol purple" and "brom thymol blue" were used as indicators for the media on the acid side; "brom thymol blue" for those concentrations around true neutrality, while in the alkaline zone, "phenol red," "cresol red," and "thymol blue" were employed. The various media were then neutralized essentially as described below for regular diphtheria toxin bouillon, the final ion concentration being determined electrometrically.

For the routine production of diphtheria toxin, in bouillon, hydrogen-ion concentrations around  $1.0 \times 10^{-8}$  ( $P_H^+ = 8.0$ ) have been employed with excellent results. Such reaction values are rapidly obtained by taking, for the preliminary adjustment, 10 c.c. of the medium, after the first heating, diluting with about 40 c.c. of distilled water, and titrating against dilute (N/10) sodium hydroxide in the cold, using phenolphthalein as the indicator. A little experience will soon determine what shade of pink should be taken as the end point. The requisite amount of strong sodium hydroxide to entirely neutralize (10N is recommended), in accordance with the preceding titration, is now added slowly and with thorough mixing to the bouillon. The medium is next heated in flowing steam for 30 minutes, or boiled for five minutes, which brings down a heavy precipitate that rapidly settles.

The reaction of the clear, supernatant liquid, or a dilution of it with "conductivity" water, is now, for very accurate work, checked by the hydrogen electrode. Ordinarily, however, a colorimetric "check" by means of the simple comparator of Hurwitz, Meyer and Ostenberg<sup>12</sup> and using standardized mixtures (of  $P_H^+ = 7.8, 8.0$ , and  $8.2$ ) with "phenol red" will be found sufficiently accurate and more rapid. In my experience, the hydrogen-ion concentration of media prepared as above, falls within a range covered by  $C_H^+ = 1.5 \times 10^{-8}$  to  $C_H^+ = 7.0 \times 10^{-9}$  and may be used for diphtheria toxin production without any further adjustment. The bouillon is now filtered hot, distributed as desired, and sterilized. All of the media used in the present investigation have been sterilized once for 20 minutes at  $115^\circ \text{C.}$  (15 pounds steam pressure).

Comparative toxicity determinations have shown that more satisfactory results are obtained by using larger flasks for cultivation of *Bact. diphtheriae* than the same proportionate amount of bouillon distributed in smaller containers. Accordingly, 3,000 c.c. of the test medium in six liter flasks were employed and inoculations made in each case, with 24 hour "starters" containing 30 c.c. of culture grown in a 250 c.c. flask to accustom the organism to the medium. After culturing for the desired length of time at  $37^\circ \text{C.}$ , the purity of the growth was checked, the samples preserved with 0.4 per cent cresols and filtered through unglazed porcelain.

In the preliminary experimentation on the ion concentration changes pro-



duced during growth of *Bact. diphtheriae*, use was made of the large flasks fitted with special syphons, so that samples could be removed aseptically from time to time as desired. Control flasks, without syphons, showed that this procedure so interfered with the growth through disturbance of pellicle formation as to entirely vitiate the value of any results obtained. A sufficient number of flasks has accordingly been employed to enable triplicate determinations of any desired value. In practically every case it has been found possible to obtain duplicate hydrogen-ion concentration and toxicity results which checked within practical limits. The toxicity valuations, made in association with my colleague, T. Ohno, were, unless otherwise noted, determined for  $L_+$  dose on samples in duplicate.

The electrometric determinations of hydrogen-ion concentration were made with the chain:

Calomel electrode ( $N$   $KCl$ ) — saturated  $KCl$  — test medium —  $Pt$  Electrode — ( $H_2$ ) at  $23^\circ C$ . The complete "set up" employed is shown in Fig. 1.  $C$  is a "normal" calomel electrode which dipped into the small vessel  $D$  containing saturated  $KCl$  to reduce to a minimum, the contact potentials between the  $N$   $KCl$  and the liquid under examination. Connection between  $D$  and the test fluid in electrode vessel  $A$  was maintained by a wick saturated with  $KCl$ , passing through a small glass syphon tube  $H$ . Hydrogen electrodes ( $B$ ) of the type described by Bovic,<sup>13</sup> were employed, supplied with purified hydrogen, generated from the electrolysis of  $NaOH$  with nickel electrodes. The temperature of the liquid in  $A$  was determined by a thermometer accurate to  $\pm 0.1^\circ C$ , and a similar thermometer was mounted near the calomel electrode  $C$ .

The hydrogen-ion concentration was read directly by means of the "ionometer" ( $E$ ) described by Bartell.<sup>14</sup> The known potential derived from a Weston Standard Cell ( $S$ ) (calibrated by the Bureau of Standards) was first brought into the circuit through the double-pole, double-throw switch  $F$  after which, sufficient resistance (4821 ohms) was thrown in to compensate for the calomel electrode employed. With the pointer arms of the logarithmic and concentration coils set at the proper values, (in this case  $3.0$  and  $10^{-13}$  respectively) the external resistances, to compensate for the storage battery, were now adjusted until no deflection was noted in the galvanometer ( $G$ ), which indicated that the instrument was in balance for direct reading. The resistance values thus obtained were found to remain practically constant for several hours with a good accumulator.

By reversing  $F$  the unknown cell consisting of hydrogen electrode and calomel was next connected in; the other resistances remaining the same, and readings could now be obtained directly in terms of hydrogen-ion concentration by manipulating the concentration and logarithmic coils until the galvanometer showed a balance. The ionometer and galvanometer were mounted so as to insure proper insulation from stray currents and freed as much as possible from tremors. During all of the measurements, the room temperature was such that no difficulty was experienced in keeping the thermometer practically constant at  $23^\circ C$ .

*Experimental Protocols.*—Before making a study of the toxicogenic and hydrogen-ion concentration changes produced by the growth of *Bact. diphtheriae* in bouillon, the question arose as to what is the optimal zone of ion concentra-

tion for such metabolic activities. This made it necessary to determine within what limits of acidity and alkalinity, a known, toxicogenic culture of *Bact. diphtheriae* can produce toxin of prescribed strength. For this purpose there were made up, as already described, lots of bouillon varying in reaction and sufficient in amount to permit of triplicate determination with large flasks at each ion concentration. The flasks were now inoculated with "starters" of a *Bact. diphtheriae*

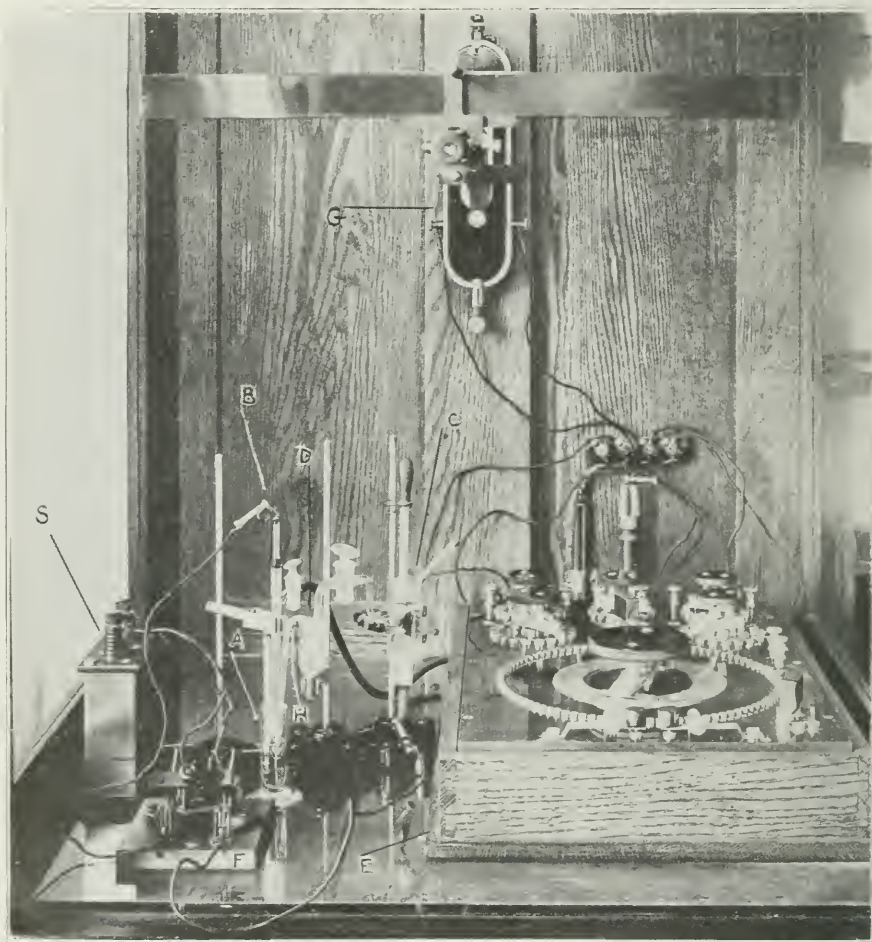


Fig. 1.

culture (No. C36) originally obtained from W. H. Park and capable of elaborating a toxin in standard bouillon of which one L. dose is less than 0.25 c.c. Cultivation of each set was carried on at 37° C. for two weeks and at the end of this period samples were removed from each flask for determination of the final hydrogen-ion concentration. The remainder of the bouillon was then preserved and set aside for toxin estimation. The results obtained are summarized in Table I.

Inspection of the data tabulated in Table I shows that the optimal zone for metabolic activity of *Bact. diphtheriae* is in the alkaline region. While good *growth* of the organism appears to be possible within hydrogen-ion concentration limits

TABLE I

THE INFLUENCE OF VARYING H-ION CONCENTRATIONS ON GROWTH AND TOXICOGENICITY OF BACT. DIPHTHERIE

INITIAL $C_H^+$	CHARACTER OF GROWTH	FINAL $C_H^+$ (2 WEEKS)	TOXICITY L <sup>+</sup> DOSE
$6.5 \times 10^{-6}$	Very scant	$4.2 \times 10^{-6}$	More than 2.0 c.c.
$1.0 \times 10^{-6}$	Moderate	$7.0 \times 10^{-8}$	Between 1.5 and 2 c.c.
$7.5 \times 10^{-7}$	Heavy	$2.1 \times 10^{-8}$	1.0 c.c.
$1.0 \times 10^{-7}$	"	$2.0 \times 10^{-8}$	0.7 c.c.
$7.0 \times 10^{-8}$	Very heavy	$1.8 \times 10^{-8}$	Less than 0.25 c.c.
$3.6 \times 10^{-8}$	" "	$1.6 \times 10^{-8}$	" " 0.25 "
$2.0 \times 10^{-8}$	" "	$9.7 \times 10^{-9}$	" " 0.25 "
$1.0 \times 10^{-8}$	" "	$7.6 \times 10^{-9}$	" " 0.25 "
$8.5 \times 10^{-9}$	" "	$7.2 \times 10^{-9}$	" " 0.25 "
$7.0 \times 10^{-9}$	" "	$6.9 \times 10^{-9}$	" " 0.25 "
$5.0 \times 10^{-9}$	" "	$5.2 \times 10^{-9}$	" " 0.25 "
$2.3 \times 10^{-9}$	" "	$3.0 \times 10^{-9}$	" " 0.35 "
$1.1 \times 10^{-9}$	Moderate	$2.3 \times 10^{-9}$	Between 0.50 and 0.6 c.c.
$8.4 \times 10^{-10}$	"	$4.1 \times 10^{-9}$	" 0.75 " 1.00 "
$7.0 \times 10^{-10}$	Scant	$1.0 \times 10^{-8}$	" 1.5 " 2.0 "
$5.3 \times 10^{-10}$	Very scant	$7.5 \times 10^{-10}$	More than 2.0 c.c.

ranging from about  $C_H^+ = 10 \times 10^{-6}$  to about  $C_H^+ = 8.4 \times 10^{-10}$ , maximum production of toxin seems to occur only where the reaction of the bouillon falls in a concentration range from about  $C_H^+ = 7.0 \times 10^{-8}$  to about  $C_H^+ = 5.0 \times 10^{-9}$ . Anti-

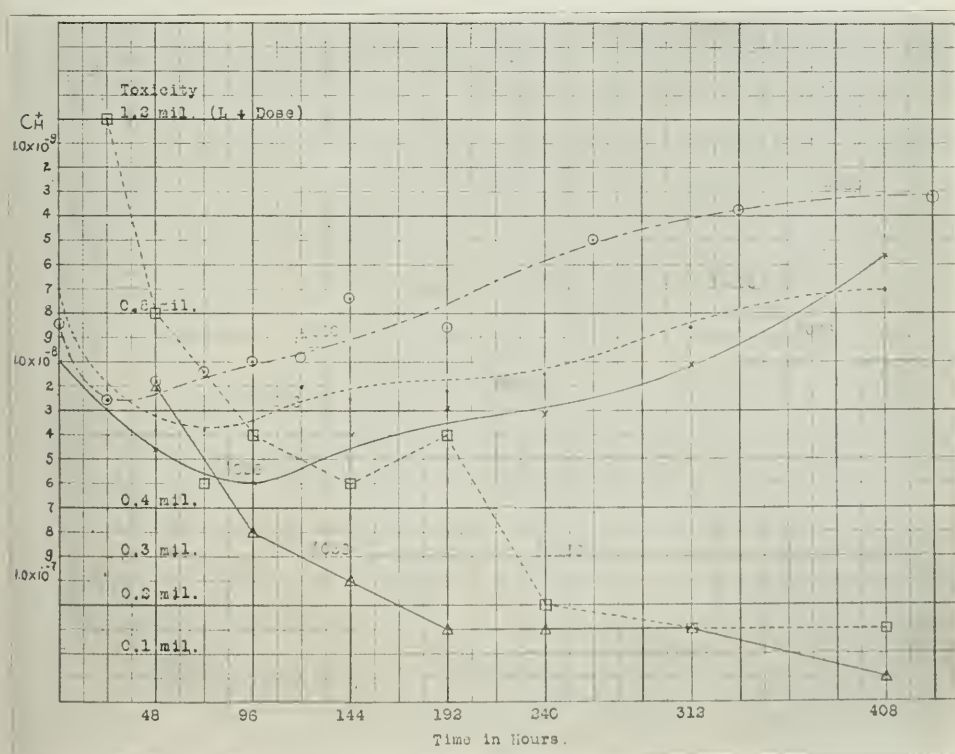


Fig. 2.



septic action, as evidenced by scant growth, is apparently exerted on the acid side around  $C_H^+ = 6.5 \times 10^{-6}$  while on the alkaline side, it does not come into consideration until about  $C_H^+ = 5.3 \times 10^{-10}$  is reached.

With the foregoing results at hand, experimentation was now undertaken to determine the changes in reaction and toxin production which take place in plain bouillon during the growth of *Bact. diphtheriæ* cultures under optimum conditions. In addition to the Park strain (No. 036) employed above for the anti-septic value test, another toxicogenic strain (No. 0236) originally obtained from McFarland, and an avirulent nontoxicogenic strain (No. 880) isolated by H. C. Ward of this laboratory were studied. The preparation of the bouillon for this purpose and the inoculation technic have been detailed in the preceding section. The accompanying curves shown in Fig. 2 have been plotted using the data obtained with the three cultures, and tabulated in Table II.

TABLE II

CHANGES IN H-ION CONCENTRATION AND TOXICOGENICITY DURING GROWTH OF *BACT. DIPHTHERIÆ* IN BOUILLON

<i>(Park)</i> Strain No. 036			<i>(McFarland)</i> Strain No. 0236			<i>(Avirulent)</i> Strain No. 880		
TIME IN HOURS	$C_H^+$	TOXICITY L+ DOSE	TIME IN HOURS	$C_H^+$	TOXICITY L+ DOSE	TIME IN HOURS	$C_H^+$	TOXICITY M.F. DOSE
0	$1.0 \times 10^{-8}$	—	0	$7.0 \times 10^{-9}$	—	0	$8.5 \times 10^{-9}$	—
48	$4.6 \times 10^{-8}$	0.65 c.c.	24	$2.1 \times 10^{-8}$	1.0 c.c.	24	$2.3 \times 10^{-8}$	No reaction
			48	$3.2 \times 10^{-8}$	0.8 "	48	$1.9 \times 10^{-8}$	2 c.c.
			72	$3.8 \times 10^{-8}$	0.45 "	72	$1.3 \times 10^{-8}$	
96	$6.0 \times 10^{-8}$	0.35 "	96	$3.4 \times 10^{-8}$	0.55 "	96	$1.1 \times 10^{-8}$	
			120	$2.1 \times 10^{-8}$	0.35 "	120	$9.5 \times 10^{-9}$	No reaction
144	$4.0 \times 10^{-8}$	0.25 "	144	$2.5 \times 10^{-8}$	0.45 "	144	$7.4 \times 10^{-9}$	2 c.c.
192	$2.9 \times 10^{-8}$	0.15 "	192	$2.3 \times 10^{-8}$	0.55 "	192	$8.3 \times 10^{-9}$	
240	$3.0 \times 10^{-8}$	0.15 "	240	$1.6 \times 10^{-8}$	0.15 "			
						264	$5.5 \times 10^{-9}$	
312	$1.2 \times 10^{-8}$	0.15 "	312	$8.7 \times 10^{-9}$	0.15 "			
						336	$3.8 \times 10^{-9}$	Necrosis 2 c.c.
408	$4.7 \times 10^{-9}$	0.05 "	408	$7.0 \times 10^{-9}$	0.15 "			
			528	$5.0 \times 10^{-9}$	0.15 "	432	$3.2 \times 10^{-9}$	Necrosis 2 c.c.

Comparison of the hydrogen-ion concentration curves of Fig. 2 shows a decided similarity with all three of the strains examined. There is, at first, a small production of acid, as shown by an increase in the concentration of hydrogen ions, which soon reaches a maximum, the amount of acid produced varying with the organism. The Park strain (No. 036) gives the maximum amount of acid, the other toxicogenic strain (McFarland No. 0236) produces nearly as much, while the avirulent strain develops only about one-half of the amount.

Inspection of Fig. 2 indicates that the slope of the acid production curves is very nearly the same for the three strains, so that the total increase in hydrogen-ion concentration directly depends on the length of time the organism undergoes acid fermentation. Strain No. 036 required four days to reach a maximum value, No. 0236 apparently had attained this point in 72 hours while No. 880 seems to have begun an alkaline fermentation after the first day. In fact, it



will be noted that all three strains show a reversal, followed by alkaline fermentation which apparently continues until an antiseptic hydrogen-ion concentration is reached.

By way of obtaining some information as to the source of the acid production in plain bouillon, two sets of media were prepared as already directed. One set consisted of the beef infusion used in the preceding experimentation with 0.5 per cent sodium chloride, and the other had 2 per cent of the peptone and 0.5 per cent of the sodium chloride. Both sets were inoculated with starters of the Park strain (No. 036) and the hydrogen-ion concentration changes occurring in each during growth were now studied in accordance with the technic already employed. The results are shown in Table III.

TABLE III

HYDROGEN-ION CONCENTRATION CHANGES DURING GROWTH OF BACT. DIPHTHERIÆ IN STRAIGHT BEEF INFUSION AND 2% PEPTONE SOLUTION

<i>Beef Infusion</i>			<i>2% Peptone Solution</i>		
TIME IN HOURS	$C_H^+$	TOXICITY M.F. DOSE	TIME IN HOURS	$C_H^+$	TOXICITY M.F. DOSE
0	$8.5 \times 10^{-9}$		0	$7.5 \times 10^{-9}$	
24	$9.4 \times 10^{-9}$	No reaction	24	$8.4 \times 10^{-9}$	No reaction
		2 c.c.			2 c.c.
48	$1.3 \times 10^{-8}$	" "	48	$1.0 \times 10^{-8}$	" "
96	$2.7 \times 10^{-8}$	" "	96	$8.7 \times 10^{-9}$	" "
168	$2.7 \times 10^{-8}$	" "	168	$7.0 \times 10^{-9}$	Slight reaction
					2 c.c.
240	$2.6 \times 10^{-8}$	" "	240	$6.5 \times 10^{-9}$	" "
408	$2.9 \times 10^{-8}$	" "	408	$6.1 \times 10^{-9}$	No reaction
					2 c.c.

It is readily apparent from the above data, that both components of plain bouillon permit of acid fermentation by Bact. diphtheriæ. As was expected, the growth in straight infusion was scant, while that in the 2 per cent peptone solution (+ salt) appeared only about half as heavy as in regular bouillon. Table III shows that in plain beef infusion, the diphtheria bacillus produces a small amount of acid which reaches a maximum in about four days, after which the hydrogen-ion concentration remains practically constant. In the peptone solution, there is also an initial acid production increasing to about the third day, but then followed by a reversal and steady decrease in hydrogen-ion concentration as in bouillon. Estimation of the amounts of acid produced in each case indicates that the sum total of both sets very nearly approximates the initial H-ion increase during growth in plain bouillon, as given in Fig. 2.

The toxicity values for No. 036 show a steady increase until the eighth day. Five days later, the value appears to be constant, but increases in toxicity at the end of seventeen days. The toxin development in the case of No. 0236 is irregular, fluctuating up to the eighth day, after which there is a rapid increase in toxicity, reaching a maximum on the thirteenth day and remaining constant at this value to the end of the experiment. It is interesting to note that the avirulent strain (No. 880) produces no toxin at first (2 c.c. dose), but sufficient is developed at the end of two weeks to cause necrosis at the site of injection with the same dose. That it is actually diphtheria toxin which is

produced, is shown by the fact that addition of antitoxin (as in testing by L+ dose) causes neutralization and no necrosis.

#### DISCUSSION

As has been found true in the case of several other organisms, notably in the colon and streptococcus groups (Michaelis and Marcora,<sup>15</sup> Ayers,<sup>16</sup> Itano<sup>17</sup>), the hydrogen-ion concentration of the medium, from the data presented above, is seen to have a decided influence on the metabolism of *Bact. diphtheriae*. While there is relatively a wide range, from about  $C_H^+ = 1.0 \times 10^{-6}$  to about  $C_H^- = 8.4 \times 10^{-10}$ , in which good growth can take place, maximal elaboration of toxin occurs only in a small zone of hydrogen-ion concentration, covered by  $C_H^+ = 7.0 \times 10^{-8}$  to  $C_H^- = 5.0 \times 10^{-9}$ . The upper limits of alkalinity very closely approximate in value those obtained by the author for the H-ion concentration of antidiphtheric serum (equine) and the lower is very near the  $C_H^+$  value given by McClendon and Magoon<sup>18</sup> for blood.

It is interesting to note from Table I that *Bact. diphtheriae* can tolerate a much greater concentration of hydroxyl ions (stronger alkaline reaction) than acid ions before a value inhibitory to growth is encountered. In all probability, the antiseptic  $C_H^+$  values obtained with the Park strain ( $6.5 \times 10^{-6}$  and  $5.3 \times 10^{-10}$ ) are of general application to *Bact. diphtheriae* since "starters" of the McFarland Strain (No. 0236) fail to develop at  $C_H^+ = 6.5 \times 10^{-6}$  and give only very scant growth in a medium reading  $C_H^+ = 5.0 \times 10^{-10}$ . No growth at all can be obtained with the avirulent strain at either of the above mentioned hydrogen-ion concentrations.

Confirming, in a general way, the results obtained by other investigators, the curves plotted in Fig. 2 show that *B. diphtheriae*, when cultivated in plain bouillon undergoes an initial acid fermentation. Contrary to what might be supposed from the introductory review, the total increase in hydrogen-ion concentration, even with the most vigorous strain (Park, No. 036) is relatively small (from about  $C_H^+ = 1.0 \times 10^{-8}$  to  $C_H^+ = 6.0 \times 10^{-8}$ ), and appears to be due to an acid fermentation of some constituent, very likely carbohydrate in nature, in both the beef infusion and the peptone. It should also be noted that even at the point of maximum acid development, the reaction is definitely alkaline.

The results given in Table I strongly indicate that an actual acid reaction ( $C_H^+ = 1.0 \times 10^{-7}$  = neutral) has a destructive action on diphtheria toxin. From the amount of acid produced by both of the above toxicogenic strains, it would seem that where the initial  $C_H^+$  of the medium is appreciably greater than  $7.0 \times 10^{-8}$ , the increase in hydrogen-ion concentration resulting from growth would be sufficient to produce a final acid reaction destructive to the toxin. This is actually found to be the case. Table I shows that where the initial reaction of the bouillon is  $C_H^+ = 7.0 \times 10^{-8}$ , a strong toxin with L+ dose of less than 0.25 c.c. is obtained. On the other hand, a slight increase in the initial reaction to  $C_H^- = 1.0 \times 10^{-7}$  is sufficient to make the final potency drop to an L+ dose of about 0.7 c.c.

The total amount of acid produced appears to be a specific property of each individual strain. The avirulent strain develops only about half as much acid as do the toxicogenic strains, the increase in hydrogen-ion concentration reaching a maximum soon after the first day in the former case. It may be probable

that this diminished capacity for acid fermentation is a general property of avirulent strains and is possibly an index of decreased metabolic activity.

The data given in Tables II and III further show that the natural course pursued by *Bact. diphtheriae* growing in plain bouillon is in the direction of increased alkali production. This decrease in hydrogen-ion concentration takes place steadily after the initial acid fermentation and reversal, and apparently continues until a limiting concentration is attained.

It is obvious from the curves plotted in Fig. II that in the normal development of *Bact. diphtheriae* in bouillon, the organism may produce the same hydrogen-ion concentration at two different intervals, which represent wide variations in potency. Other than the fact that it is necessary to have the initial reaction of the bouillon within the alkaline limits above mentioned, and that potent toxin will have an alkaline reaction, the experimental data indicate that there is no direct relationship during growth between the hydrogen-ion concentration of the medium and the production of toxin.

In conclusion, I desire to express my most sincere thanks to Professor F. E. Bartell of the University of Michigan for valued advice on the determination of hydrogen-ion concentration.

#### CONCLUSIONS

1. Toxin of maximum potency is produced in bouillon by *Bact. diphtheriae* only when the initial reaction falls within a certain zone of alkalinity, included within the hydrogen-ion concentration limits of about  $7.0 \times 10^{-8}$  to about  $5.0 \times 10^{-9}$ . Luxuriant growth of the organism appears to be possible where the reaction of the bouillon ranges from about  $C_H^+ = 1.0 \times 10^{-6}$  to about  $C_H^+ = 8.4 \times 10^{-10}$ .

2. When cultivated in plain bouillon under optimal conditions, *Bact. diphtheriae* undergoes an initial increase in hydrogen-ion concentration. This is soon followed by a steady decrease until apparently a limiting alkaline reaction is attained. The total acid produced is relatively small and seems to vary in amount with each individual strain. Toxicogenic strains appeared to develop more acid than an avirulent strain. The initial increase in hydrogen-ions is due to fermentation of some constituent in both peptone and beef infusion.

3. No direct relationship can be found between the hydrogen-ion concentration of the medium and toxicity during the growth of *Bact. diphtheriae*.

#### BIBLIOGRAPHY

- <sup>1</sup>Roux and Yersin: *Ann. de l'Inst. Pasteur*, 1888, 1889, 1890, 1894.
- <sup>2</sup>Spronck: *Ann. de l'Inst. Pasteur*, 1895, ix, 758; *Ibid.*, 1898, xii, 700.
- <sup>3</sup>Park and Williams: *Jour. Exper. Med.*, 1896, i, 164.
- <sup>4</sup>Madsen: *Ztschr. f. Hyg. u. Infektionskrankh.*, 1897, xxvi, 157.
- <sup>5</sup>Smith: *Jour. Exper. Med.*, 1899, iv, 373.
- <sup>6</sup>Hitchens: *Jour. Med. Research*, 1904-5, xiii, 523.
- <sup>7</sup>Lubenau: *Arch. f. Hyg.*, 1908, lxi, 305.
- <sup>8</sup>Jacobsen: *Centralbl. f. Bakteriöl.*, 1910-11, lvii, Part 1, p. 16.
- <sup>9</sup>Clark: *Jour. Infect. Dis.*, 1915, xvii, 129.
- <sup>10</sup>Davis: *Jour. Lab. and Clin. Med.*, 1917, iii, No. 2, p. 75.
- <sup>11</sup>Clark and Lubs: *Jour. Bact.*, 1917, ii, Nos. 1, 2, 3, pp. 1, 109, 191.
- <sup>12</sup>Hurwitz, Meyer and Osterberg: *Bull. Johns Hopkins Hosp.*, 1916, xxvii, 17.
- <sup>13</sup>Bovic: *Jour. Med. Research*, 1915, xxxiii, 295.
- <sup>14</sup>Bartell: *Jour. Am. Chem. Soc.*, 1917, xxxix, No. 4, p. 630.
- <sup>15</sup>Michaelis and Marcora: *Zeitschr. f. Immunitätsforsch. Exper. Ther.*, 1912, Orig., xiv, 170.
- <sup>16</sup>Ayers: *Jour. Bact.*, 1916, i, 84.
- <sup>17</sup>Itano: *Bull. Mass. Agr. Exper. Sta.*, 1916, No. 167.
- <sup>18</sup>McClendon and Magoon: *Jour. Biol. Chem.*, 1916, xxv, No. 3, p. 669.

# LABORATORY METHODS

---

## NOTE ON THE COLLOID CHEMISTRY OF FEHLING'S SUGAR TEST\*

BY MARTIN H. FISCHER, M.D., AND MARIAN O. HOOKER, M.D.,  
CINCINNATI, OHIO

AS familiarly known, especially by the clinicians, the reduction of Fehling's alkaline copper solution to cuprous oxide by sugars and certain other reducing materials presents variations, the nature of which is not yet fully understood. In testing the urine of diabetics for dextrose, for example, it is usually expected that the presence of sugar will betray itself through a reduction of the copper salt to a bright red precipitate of copper oxide. It frequently happens, however, that the reduction does not yield this bright red result, but a more orange, or distinctly yellow precipitate; while, under certain circumstances, only a greenish discoloration of the originally bright blue solution is obtained, from which, at the best, a light greenish or dirty yellow precipitate may settle out in the course of many days.

The explanations which have been given of these findings are different with different authors, but they are, for the most part, chemical in nature. The appearance of the green color, with little tendency to form a precipitate, is often regarded as entirely questionable evidence of the presence of dextrose (or other reducing material). The yellow precipitate is usually accepted as clear proof for the presence of a reducing body (like dextrose) but this yellow substance is not considered identical chemically with the red cuprous oxide. Many, for example, hold the yellow precipitate to be a hydrated form of the copper oxide.

Because of this confusion regarding the nature and the interpretation of the reaction, we decided to examine it from a colloid-chemical point of view, for we felt in advance that the explanation of what was really at the bottom of these apparent differences in end results was more likely to be found in these regions than in the more commonly studied ones of pure chemistry. We felt that *Fehling's reduction test, under different circumstances, does not yield chemically different copper oxides, but one and the same copper oxide in different degrees of subdivision* (possessed of different degrees of dispersion).

That one and the same chemical compound (as a metal, a sulphide or an oxide) may, in the colloid state, show different colors has long been known to different workers.<sup>1</sup> It has, however, remained for Wolfgang Ostwald<sup>2</sup> to recognize that these color variations tend, on the whole to follow a general order and that this order is coordinate with the size of the dispersed particles. The most highly dispersed particles of a given substance are likely to be yellow; as their size increases, they become orange, then red, and finally violet, blue, or black.

\*From the Eichberg Laboratory of Physiology in the University of Cincinnati.





Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fischer and Hooker:  
"NOTE ON THE COLLOID CHEMISTRY OF FEHLING'S SUGAR TEST"



When the ordinary, brilliantly blue Fehling's solution is mixed with a little dextrose solution or some diabetic urine and the mixture is not boiled, as ordinarily, but is simply allowed to stand a number of hours at room temperature, a series of such color changes as are represented in Figs. 1 to 5 may be observed. The originally clear Fehling's solution early loses its brilliancy and assumes the more opaque blue green color of Fig. 1. It soon passes from this to the leaf green of Fig. 2 and then to the yellow green of Fig. 3. At this time more or less yellow precipitate may separate out. Later, not only this yellow precipitate, but the supernatant liquid as well become yellow orange, then bril-



Fig. 6.



Fig. 7.

liantly orange as in Fig. 4 and finally the bright red shown both by the solution and precipitate of Fig. 5..

It is of interest now to follow the microscopic appearances which are coincident with these macroscopic optical changes. This may be done by examining drops of fluid from different tubes as the different colors are developed, or the whole series may be observed in one and the same drop as the reduction progresses under the microscope. Examined microscopically or ultramicroscopically, the original Fehling's solution is optically homogeneous. If a drop of the

fluid from such a less brilliant tube as is shown in Fig. 1 is examined, one early becomes conscious of a vague blurring in the microscopic field. By waiting a little, this is seen to be followed by a brilliant lighting-up of the field through vast numbers of actively motile particles. A photograph taken about this time yields Fig. 6. We may now examine drops of fluid from the yellow, orange, or red tubes or we may simply watch the original preparation under the microscope as this passes, successively, through these same color changes. As shown in Figs. 7 and 8, the particles gradually grow in size so that when they appear distinctly yellow and start to precipitate as in Fig. 3, the microscopic picture shown in Fig. 7 is observed. When the brilliant red precipitate is reached, the microscope reveals particles of the size shown in Fig. 8.

These observations show, therefore, that *the different colors observed in the reduction of Fehling's solution by dextrose (or other reducing substances) are nothing more than color changes coincident with a gradual increase in the size of copper oxide particles.*

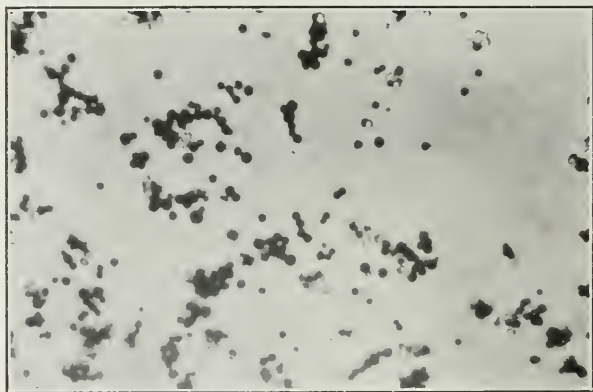


Fig. 8.

To the practical chemist who, like the clinician, uses Fehling's test in order to determine qualitatively or quantitatively the presence of reducing substances in any fluid (like urine), the simple observations detailed in this paper are not only useful, but they make clear the meaning of several things which have previously been ambiguous.

These observations show that any of the positive reactions here described (whether they yield merely a dirty green solution, a yellow or orange precipitate, or the brilliantly red precipitate so generally sought) indicates equally well the presence of a reducing substance. A dirty pea-green solution, in other words, means just as much from a qualitative point of view as does a red precipitate. Most textbooks would have it that this is not the case, or, if they admit that such a reaction is important qualitatively, they hold that it means the presence of but little reducing material. It is of interest, therefore, to emphasize that in the case of dextrose, for example, the presence of much of this material is quite as likely, in fact more likely, other things being equal, to yield merely a greenish discoloration or a yellow precipitate than is a lesser amount. In fact, to



insure getting the red precipitate, one must in all instances use *little* enough of the reducing material. Generally speaking, amounts of dextrose too small to reduce all the copper salt present will more definitely yield a red or an orange precipitate than larger amounts.

This fact, as well as certain others to be discussed immediately will, of course, be readily intelligible to the colloid chemist. With much sugar present, the number of points at which the copper salt is attacked and reduced will evidently be much larger than when less sugar is added. All the available copper salt for further growth of the particles will, therefore, have been exhausted when the copper oxide particles are still small, wherefore the presence of too much reducing substance is more likely to yield only the allegedly ambiguous end reaction than when less is used.

Another factor must, however, be considered as active in determining what the end result will be. Obviously, if the reduced copper oxide can be stabilized anywhere in its progress from the finely divided material to the coarse red, then one of the intermediate colors is obtained. As is also familiar to the colloid chemist, such stabilization of a finely divided suspension colloid is commonly brought about through the presence in the reaction mixture of various hydrophilic (lyophilic) colloids.

The truth of this general conclusion may be tested, not only experimentally with the pure materials of the chemical laboratory, but it is done daily in the clinicochemical experience of the laboratory worker in medicine. If to the dextrose solution used to produce such a series of color reactions as is shown in Figs. 1 to 5 we add some mucin, some acacia, some gelatin, a little egg-white or some other hydrophilic colloid, the rate at which the various colors is obtained is much delayed. In fact, if the concentrations are chosen properly, only dirty green or yellowish green reductions may be obtained, no matter how much copper oxide reducing substance may be present. The copper oxide may, in other words, be stabilized in any of its various states of subdivision.

What has been written above might seem at first sight not to harmonize with the practical fact, well known to the clinicians, that the urine of a severe diabetic will more often give a more perfect (red) reduction of Fehling's solution than will the urine from a milder diabetic or that from a normal individual in whom an alimentary glycosuria has been produced by excessive consumption of carbohydrate. The reasons for this are to be found in the fact that a severe diabetic with high dextrose elimination is usually also the victim of a polyuria. His urine while high in reducing substance (and consequently by itself inclined to yield a green or yellow result) is, therefore, relatively poor in those protective substances which give normal (nonpolyuric) urine its restraining powers for the stabilization of the copper oxide particles when these are still small. Conversely, the urine of a more nearly normal individual (containing, for example, but little dextrose following an excessive carbohydrate ration) will, it is true, contain but little reducing substance, but what makes for the stabilization of the copper oxide in its finely divided form is the large content of protective materials present.

The scientific basis of the old trick of diluting heavily the material to be examined whenever such questionable reductions are obtained is easily seen.

Dilution not only dilutes the highly concentrated reducing substance, but, more important, it dilutes the stabilizing colloids to a point where their powers in this direction are largely lost.

To these factors of the concentration of the reducing substance itself and the concentration of the various protective colloids which may be present comes a third factor which determines the nature of the end result. When dextrose, for example, is treated with an alkali, a series of degradation products is formed, as is well known, and it is these which in their turn are the elements directly responsible for the reduction of the copper salt. In the formation of these degradation products, however, some are produced which are in themselves colloid and hydrophilic in type. When, therefore, too much of a solution containing dextrose or any similar reducing substance is added to Fehling's solution, there appears the danger of getting intermediate colloid substances which in themselves tend to stabilize the copper oxide before it has attained the coarse dimensions necessary to yield an orange or red precipitate.

A final word must be added regarding the effects of temperature in the production of the red precipitate in a reducing Fehling's solution. Reduction at high temperature (as in the usual procedure) is more likely to yield the colloid types of reduction products than lower ones (like room temperatures). A urine containing an amount of reducing body which upon boiling yields only a dirty green solution, will show a bright red precipitate if the same proportions of urine and Fehling's solution are simply mixed and set aside at room temperature for twenty-four hours. The reasons for such improvement are probably several, but the most important would seem to be the initiation of the reduction at fewer points under the latter circumstances, while, because of the greater time element, these fewer points would then be given better opportunity to grow to the sizes characteristic of the red precipitate.

#### ADDENDUM

Since writing the above and since the appearance of a preliminary note<sup>3</sup> discussing this subject, Stanley Benedict has called our attention to the fact that many of the points made in our communication have been previously recognized by Hugh MacLean.<sup>4</sup> MacLean, in 1906, questioned the chemical nature of the differences in the results obtained upon reducing Fehling's solution and brought forth evidence which he felt indicated that these differences were merely physical. While his proofs have not been accepted as final by Benedict, we think, in the light of the additional facts detailed above and the clearer notions now available than then regarding the essential nature of the colloid state, that MacLean's general conclusions may be accepted with entire safety.

Failure to get a red precipitate MacLean holds to be due to something which keeps the cuprous oxide "in solution." If the word "solution" is not interpreted too strictly, but in the way in which it was used a decade ago when we still felt free to speak of liquid colloid suspensions as "solutions," then no objection must be raised to this contention of the author. As a matter of fact, in developing his theme he shows that what he means by "solution" is a suspension of particles of different sizes. He demonstrates these differences by showing that the green, the yellow, the orange, and the red liquids, or pre-

precipitates, pass with increasing difficulty through filters of known porosity. But this filtration method as we now know constitutes one of the very schemes employed by colloid chemists in order to determine (somewhat roughly) the size of particles in colloid suspensions. Finally, in trying to say what it is, in urine for example, which keeps the cuprous oxide "in solution" (or, in more modern terms, in a state of high dispersion) he fixes first attention upon the presence of creatinine. MacLean holds that this substance (rather specifically it would seem from the contentions of the author) keeps the cuprous oxide "in solution." Considering the fact that any hydrophilic colloid which is not dehydrated by the conditions of the experiment will retard or prevent the development of the cuprous oxide to its red form, a specific action is probably not to be assigned to the creatinine. As MacLean himself points out, the creatinine by itself "discolors" but does not reduce Fehling's solution. By removing some of the available copper, addition of the creatinine, therefore, substitutes for the ordinary mixture in which the amount of copper salt exceeds the amount of the reducing body, one in which the opposite conditions obtain. This, therefore, by itself, tends to give the yellow or green result. Beyond this the creatinine may form new and colloid compounds with some of the constituents of the Fehling's solution from which a general colloid protective action as detailed above would then result.

## BIBLIOGRAPHY

- <sup>1</sup>The Svedberg: Herstellung Kolloider Lösungen, Dresden, 1909, where detailed references to older observations may be found.
- <sup>2</sup>Wolfgang Ostwald: Kolloid-chem. Beihefte, 1911, ii, 409; Theoretical and Applied Colloid-Chemistry, Translated by Fischer, 1917, New York, p. 62.
- <sup>3</sup>Fischer, Martin H., and Hooker, Marian O.: Science, 1917, xlv, 505.
- <sup>4</sup>MacLean, Hugh: Brit. Med. Jour., 1907, i, 1471.

## ON THE COLLOID-CHEMICAL MIMICRY OF CERTAIN ENZYMATIC REACTIONS\*

BY MARTIN H. FISCHER, M.D., AND MARIAN O. HOOKER, M.D.,  
CINCINNATI, OHIO

**I**N the course of experiments in which Fehling's solution was reduced by different substances, we encountered a series of reactions which are so strikingly analogous to certain reactions observable in living protoplasm that we have used these reactions for class demonstration purposes. While various aspects of the phenomena to be described are, since Bredig's experiments, well known to physical chemists, their sum total is somewhat new, and having proved of teaching value in physiologic discussion we publish them here with the feeling that they may possibly prove of interest to a wider circle of laboratory workers.

As well known, formaldehyde will reduce at room temperatures a Fehling's

\*From the Reichberg Laboratory of Physiology in the University of Cincinnati.

solution not only to the ordinary cuprous oxide, but to the metallic copper. As the copper begins to be formed it comes down in a finely divided state, in other words, in colloid form. As soon, however, as this colloid copper appears, a second reaction ensues. The metallic copper begins to act upon the formaldehyde and to decompose this with the liberation of a gas. The gas is hydrogen. When this experiment is done at ordinary room temperatures, when the Fehling's solution and the formaldehyde are first mixed (in the proportion of 10 c.c. of 40 per cent formaldehyde to 90 c.c. of Fehling's solution), nothing apparently happens; but, after some hours the bright blue color becomes less transparent as a consequence of the gradual formation of the metallic copper, and, as this ensues, tiny bubbles arise throughout the fluid so that the whole mixture becomes distinctly effervescent. This process continues until all the formaldehyde has been decomposed or until all the blue color disappears and nothing is left behind but a spongy copper, from which there still rise occasional bubbles of liberated gas.

When biologic terms are applied to the set of reactions here described, we may say that, from the mixing together of a metallic salt, an alkali and a simple carbohydrate, we observe, first, the production of an enzyme (the colloid copper). In other words, there results from mixing together a series of very simple "dead" substances the production of that most characteristic of all the substances which characterize "living" matter. Looked at another way, the Fehling's solution represents a reaction mixture which is "injured," "poisoned" or destroyed by the formaldehyde. But against this "poison" or "toxin" the reaction mixture produces an "antitoxin" (the reduced copper), which in its turn decomposes the formaldehyde and so retards or prevents its further injurious action upon the Fehling's solution.

The analogy to biologic reactions is further heightened when it is pointed out that the presence of various substances will not only prevent the formation of the enzyme (the reduced copper), but will inhibit its action after formation. Potassium cyanide (which according to the classical findings of Geppert is particularly active in this regard in biological reactions, and according to the findings of Bredig, in the case of the colloid inorganic "ferments") shows marked action upon the decomposition of Fehling's solution by formaldehyde. Whether the cyanide be added to an original mixture of Fehling's solution and formaldehyde, or whether it be added after the copper reduction and the secondary decomposition of the formaldehyde have been well established, presence of the cyanide inhibits or stops entirely further decomposition in the reaction mixture.

The decomposition of formaldehyde under the influence of colloid copper with the production of hydrogen may also be used to illustrate much of what is considered fundamental in respiration. As emphasized in the classic views of Hoppe-Seyler, the production of nascent hydrogen is held to be essential in the chemistry of biologic oxidation. But depending upon whether this production of the hydrogen occurs in the presence or in the absence of oxygen, totally different effects (as an oxidation in the one case or a reduction in the other) may be brought about.

If to a fresh mixture of Fehling's solution and formaldehyde, or to one



in which partial reduction to metallic copper has already occurred, is added a reducible substance, it is found that, if oxygen is admitted to the reaction mixture, the reducible substance is not affected, while it is deoxidized if the oxygen is shut off. Such dyes as methylene blue or phenolsulphonephthalein which, with loss of oxygen yield colorless compounds, work best in this regard. Dyes which are easily distinguishable from the blue of Fehling's solution are naturally to be preferred. The red of phenolsulphonephthalein is, therefore, particularly good. If some of this dye is added to a mixture of Fehling's solution and formaldehyde (150 c.c. Fehling's solution plus 15 c.c. 40 per cent formaldehyde plus 0.6 gram phenolsulphonephthalein) and the resulting solution is divided, one-half being poured into a tall test tube in which it is but little exposed to oxygen, while the other half is kept in a flat dish with a large surface exposed to oxygen, it is observed that the red color of the phenolsulphonephthalein is lost after some hours in the tube protected against the air, while it remains unchanged in the open dish. The nascent hydrogen, in other words, unites in the latter instance with the oxygen of the air and leaves the dye unaffected, while in the absence of atmospheric oxygen the hydrogen seizes upon the oxygen of the phenolsulphonephthalein and decolorizes it.\* The phenolsulphonephthalein can be shown to be still present in the tall test tube and in a readily oxidizable state. It is only necessary to shake the tube a little so as to get some of the atmospheric oxygen dissolved in the upper layers of liquid or to pour the whole reaction mixture into a second test tube through the air, to see the bright red color of the phenolsulphonephthalein reappear.

We see in these reactions an analogy to those upon which Hoppe-Seyler laid much stress and an illustration of the important differences so long recognized in biochemical reactions occurring in the presence of oxygen or in its absence. If we regard phenolsulphonephthalein as the analogue either of a protoplasmic "food" or of a protoplasmic "poison" to that "fermentation" mixture which we have likened to a biologic respiration system, it is obvious that the fate of the "food" or "poison" and hence of its effects upon the substrate, is something totally different in the presence and in the absence of oxygen. The same respiratory "ferments" produce effects in the one case which are totally lacking in the other.

\*The behavior of the methylene blue is particularly interesting because it is the substance upon which Paul Ehrlich made his fundamental studies in tissue oxidation. Phenolsulphonephthalein is of interest because it is this dye which has found such extensive use in various functional tests for kidney efficiency. We have repeatedly emphasized (Martin H. Fischer, *Oedema and Nephritis*, New York, 1915, ed. 2, 621) the great care necessary in the interpretation of all functional tests, especially when such are used to demonstrate functional incapacity. In the specific case of the phenolsulphonephthalein test for kidney efficiency, for example, not only must it be remembered that a normal dye output can still be obtained, even when out as little as one-fourth to one-eighth the total kidney substance is still intact, but with any amount of normal kidney tissue various extrarenal factors in no way associated directly with the kidney state may make for a too low dye output even though the kidney itself is not primarily at fault. Anything which increases the adsorptive power of the body colloids for the phenolsulphonephthalein, for example, will work in this direction. In this way the dye is held in the body tissues, and, failing to get to the kidney, a low phthalein output results. E. B. Reemelin (*Lancet-Clinic*, 1916, cxv, 327) and Raphael Isaacs (*Am. Jour. Physiol.*, 1916, xlii, 163) have shown that every increase in the acid content of the colloids of the blood—in other words, in all states in which there is an abnormal production or accumulation of acids in the body—will make this hold fast the injected dye and so it fails to come out in the kidney even when this organ is normal. But what is of especial interest in connection with the experiment detailed above is that it was devised to demonstrate in an ordinary physicochemic reaction mixture, the observation of E. C. Kendall (*Am. Jour. Physiol.*, 1917, xlii, 522) that phenolsulphonephthalein is reduced to a colorless compound by tissue pulp whenever oxygen is absent, while this reduction fails to be made in its presence. Failure to excrete phenolsulphonephthalein may mean, therefore, not only a loss of kidney function to below the last quarter or eighth of its functional capacity, but a state of high general acid content, or an absence of oxygen, or these several factors together.

## THE MASTIC TEST FOR THE DIAGNOSIS OF CEREBROSPINAL SYPHILIS

BY FLETCHER LANGDON, M.D., CINCINNATI, OHIO.

THE mastic test was published in 1915, by Emanuel, in an effort to find a simple and reliable test for the diagnosis of syphilis in the cerebrospinal fluid.

This test depends on the precipitation of a solution containing gum mastic, in cases of syphilis, and no change in the solution in negative cases.

The method in use was devised by J. A. Cutting\* of the Agnew's State Hospital, Agnew, California, and is as follows: A stock mastic solution is made consisting of 10 grams of pure gum mastic in 100 c.c. of absolute alcohol; this is filtered and kept in tightly stoppered bottles in which it will keep indefinitely. One part of this stock solution is added to nine parts of absolute alcohol and the mixture is then insufflated into forty parts of distilled water, producing an opalescent solution which keeps well for several days and is the solution used in the actual test.

The absolute alcohol used in the test should be of highest purity and not merely dehydrated alcohol. Make a 1.25 per cent solution of sodium chloride (NaCl) in distilled water and to this add 1 c.c. of a 0.5 per cent solution of potassium carbonate for each 100 c.c. of NaCl solution used.

Six test tubes are placed in a rack; in the first tube is placed 0.5 c.c. of the spinal fluid, to be tested, and 1.5 c.c. of the combined 1.25 per cent NaCl and 0.5 per cent potassium carbonate; place in tubes Nos. 2, 3, 4, 5, and 6, one c.c. each of the 1.25 per cent NaCl and 0.5 per cent potassium carbonate solution. Remove, with a 1 c.c. bulb pipette, 1 c.c. of the mixture from the tube No. 1 and place in No. 2, and from No. 2 to No. 3, etc., until tube No. 5 is reached. The spinal fluid mixture (1 c.c.) is discarded from tube No. 5; tube No. 6 is the control tube and receives none of the spinal fluid mixture.

Mix the fluid in the test tubes by shaking or stirring with a clean glass rod. Add 1 c.c. of the mastic emulsion to each of the six tubes and mix again thoroughly. Allow to stand in a warm place (room temperature) for from twelve to eighteen hours, or incubate at 37° C. for six to twelve hours or incubate at 37° C. for two hours and centrifuge before reading.

In positive cases the mastic will be precipitated in the first one, two, three, or four tubes (or higher in strongly positive cases) leaving the supernatant fluid clear and the mastic a white, flocculent precipitate at the bottom of the tube, though in some cases the opalescent appearance of the fluid remains the same, with, however, a fine white precipitate of mastic at the bottom of the tube. The control should never change its appearance.

In the serologic laboratory of the Cincinnati General Hospital we tested by this method 101 specimens of cerebrospinal fluid. These specimens were, whenever possible, submitted also to the Wassermann, gold curve, globulin (butyric acid test) and a blood Wassermann test.

The following table shows what results were obtained. These 101 speci-

\**Jour. Am. Med. Assn.*, June 16, 1917.

mens were not selected, but came to the laboratory for the routine serologic examination (Table I).

TABLE I\*

CEREBROSPINAL FLUID		MASTIC			GOLD CURVE				GLOBU-LIN		BLOOD WASS.		No Gold Curve	No Globulin	No Blood Wassermann
		Positive	Negative	Doubtful	Paretic	Luetic	Meningitic	Negative	Positive	Negative	Positive	Negative			
Positive Wassermann	31	26	3	2	21	4	2	1	26	2	13	2	3	3	16
Negative Wassermann	62	6	54	2	3	13	1	39	4	52	0	9	6	6	53
Doubtful Wassermann	8	1	7	0	0	3	0	5	3	5	1	2	11	2	0
Gold curve paretic	22	16	6	0	0	0	0	0	23	1	11	2	0	0	0
Gold curve meningitic	3	2	1	0	0	0	0	0	3	0	0	0	0	0	0
Gold curve luetic	18	8	9	1	0	0	0	0	15	5	2	1	0	0	0
Gold curve negative	49	2	46	1	0	0	0	0	1	14	1	10	0	0	0
Globulin negative	51	2	50	1	1	5	0	47	0	0	1	10	0	0	0
Globulin positive	41	29	11	1	23	15	3	1	0	0	13	3	0	0	0

\*Owing to the fact that a number of specimens of cerebrospinal fluid contained blood, it was impossible to make the gold curve and globulin tests in every case. It was also impossible to obtain specimens for a blood Wassermann in over half of the cases.

After completing these 101 mastic tests in connection with the other routine serologic tests, our conclusions were:

1. That the mastic test, in our hands, is probably not as delicate or reliable as Lange's colloidal gold test (gold curve) properly performed.

2. That the gold curve, properly performed, will indicate whether the syphilis in the cerebrospinal fluid is of the paretic, luetic, or meningitic type; while the mastic test merely shows a positive or negative result.

3. That the colloidal gold solution is but little more difficult to make than the mastic solution when one uses care to obtain a high-grade chloride of gold for use, and that the colloidal gold solution may be made in comparatively large quantities and will keep until used.

4. That, while the gold curve requires twice the number of test tubes for each test and also double distilled water, the mastic test requires pure absolute alcohol (expensive and difficult to obtain at present) and that the mastic glassware is much more difficult to clean than the gold curve glassware and it is of primary importance that all glassware used in either test be absolutely clean, the gold curve glassware being cleaned with soap and water, then with sulphuric acid, then rinsed in distilled water and dried. The mastic ware must be cleaned with soap and water, then dried and thoroughly rinsed in strong alcohol to remove the mastic adhering to the glass.

I am under great obligations, in this work, to Dr. Oscar Berghausen, in charge of the serologic laboratory of the Cincinnati General Hospital (where this work was done) and to Mrs. Mary Rivers, the assistant demonstrator in serology under the Moos fund, and to Dr. Foy Payne for much help and kindness during the course of this work.

## AN IMPROVED METHOD FOR ANESTHETIZING ANIMALS\*

BY JOHN A. HIGGINS, ST. LOUIS, MO.

A METHOD for administering ether to animals, which I have seen quite often in the laboratory, is described by Jackson.† This method may be satisfactory if properly executed, but the following method seems much safer and can easily be handled by one man.

The apparatus used in this method (see figure) consists of an empty one-pound ether can cut in halves, of which the upper half is here used. The front end of the ether can (*A*) is perforated with numerous holes and allows perfect ventilation. Around the lower, or open, edge of the can (*B*) are also perforated numerous holes, to which is fastened much cotton covered with gauze, allowing only a sufficient opening for the average dog's nose and mouth to enter. On the top of the ether can (*C*) is a cup-shaped piece of metal in which ether is poured and allowed to enter the inside of the ether can. On each side of the ether can (*D*) are fastened wire lugs to which are fastened short stout straps and buckles.

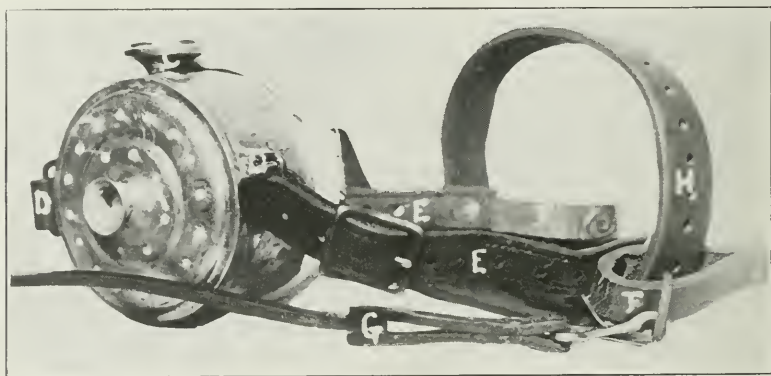


Fig. 1.

This section just described is used in the fashion of a muzzle and is held in position on the animal by straps *E* which in turn, are riveted onto the inside of straps *F*. Straps *F* are stout, about three inches long, and both ends are riveted together after placing about an inch in diameter ring on each strap. Strap *H* is an ordinary stout collar strap placed around the neck of a dog just tight enough to prevent the dog from slipping his head through the collar.

On the far ring at strap *F* is fastened a strap long enough to go around a solid stationary block or post, and fastened onto the ring on the near side at strap *F*. A snap catch may here be used as illustrated. Previous to use, the ether can should be lined with a layer of cotton.

After arranging this apparatus on a dog, the dog is set in a resting erect position on the floor. Grasp the dog's forepaws with the left hand, with the

\*From the Department of Pharmacology, Washington University Medical School, St. Louis.

†D. E. Jackson: Experimental Pharmacology, C. V. Mosby Co., 1917, p. 77.



right knee pressing down on the dog's back to keep him in place. The ether is then poured in cup *C* with the anesthetist's right hand. When the dog is well anesthetized, remove the apparatus by loosening strap *H*, place the dog on the operating table and proceed as usual.

Where a very strong dog is to be anesthetized it might be advisable to use, in connection with the previously described apparatus, a board platform set on the floor and large enough for the operator and dog to stand upon. On the rear end of the board platform should be fastened two strong upright pieces to which the dog's hind quarters are securely fastened. The dog's hind quarters should be fastened just above the middle joint of the legs and while he is in a standing position. Where the platform procedure is used, it will eliminate holding the dog in position with the right knee as previously described. This last procedure of holding a dog could be used to great advantage in the laboratory where hypodermic injections of drugs or serums, as in sensitizing anaphylactic dogs, are required.

# *The Journal of Laboratory and Clinical Medicine*

VOL. III.

MARCH, 1918

No. 6

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.  
Ann Arbor, Mich.

## ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D.	- - -	ST. LOUIS
HANS ZINSSER, M.D.	- - -	NEW YORK
PAUL G. WOOLLEY, M.D.	- - -	CINCINNATI
FREDERICK P. GAY, M.D.	- - -	BERKELEY, CAL.
J. J. R. MACLEOD, M.B.	- - -	CLEVELAND
ROY G. PEARCE, M.D.	- - -	CLEVELAND
ROGER S. MORRIS, M.D.	- - -	CINCINNATI
GERALD B. WEBB, M.D.	- - -	COLORADO SPRINGS
E. E. SOUTHARD, M.D.	- - -	BOSTON

Contents of this Journal Copyright, 1918, by The C. V. Mosby Company—All Rights Reserved  
Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

## EDITORIALS

### *The Nervous Mechanism in Thyroid Secretion*

SEVERAL years ago Professor Asher, of the University of Bern, demonstrated an experiment which was interpreted as proving the existence of a nervous mechanism to the thyroid glands. The thyroïdal nerves were stimulated with induced electric shocks of low intensity, and the response of the heart to subminimal vagus stimulation was determined at stated intervals during the experiment. If the stimulus became effective in slowing or inhibiting the heartbeat, it was taken as proof that the thyroid had produced an extra amount of thyroïdal hormone which rendered the nerve endings of the vagus hypersensitive. Here, then, was experimental proof that the secretion of the thyroid gland exalts the excitability of nerves, and that the thyroid secretion is determined by a nervous mechanism. The surgeon found in these results a reason for the removal of the glands in Graves' disease, where there is marked nervous excitability.

Unfortunately the results of many physiologic experiments are not sufficiently well defined to satisfy the critic, and Asher's work was not widely accepted by physiologists.

Some years later Cannon<sup>1, 2</sup> and his pupils at Harvard produced apparently direct evidence for the existence of secretory nerves to the thyroid. One method they employed for showing a nervous mechanism in thyroid excretion in cats consisted in fusing the anterior root of the phrenic nerve with the cervical sympathetic, and noting its effect on the thyroid function. These workers state that symptoms resembling those of Graves' disease in man developed. There were marked tachycardia, loose movements of the bowels and falling of the hair. The animals were unusually excitable. The basal metabolism was very markedly increased. The pupil was larger on the operated side, and in one of the animals exophthalmus and respiratory hippus developed on the operated side. They found that in one animal the removal of one of the glands stopped the progress of the disease, and the cat lived for seven months, when it was purposely killed. The other animals lived less than three months after the onset of the symptoms.

Troell<sup>3</sup> and Burget<sup>4</sup> have repeated the experiments of Cannon with negative results, and Marine, Rogoff, and Stewart<sup>5</sup> have just published the results of experiments on ten cats in which the operation made by Cannon was repeated. These observers failed to obtain the results reported by Cannon and his pupils in any of their series of experiments. In animals where there can be no doubt that the anastomosis was successful, they failed to find any evidence of impulses coming from the respiratory center as shown by a rhythmic change of the iris synchronous with the respiration. Exophthalmus and loss of weight were not present, nor was there any evidence of thyroid enlargement.

Such contrary results coming from men of recognized research ability are hard to understand. A significant and important clinical observation, bearing on the relationship of nerve strain and the incidence of Graves' disease was reported to the editor by Hoover, late Major in the United States Medical Reserve Corps. He states that, so far as he observed and could ascertain, while on duty in France, no cases of Graves' disease were found among the soldiers which could be attributable to the strain of the life incident to active warfare. Certainly if Graves' disease is the result of nerve strain, as some claim, it should be present in a large number of cases at the fighting front.

#### BIBLIOGRAPHY

<sup>1</sup>Cannon, Binger and Fitz: *Am. Jour. Physiol.*, 1914, xxxvi, 363.

<sup>2</sup>Cannon and Fitz: *Ibid.*, 1916, xl, 126.

<sup>3</sup>Troell: *Arch. Int. Med.*, 1916, xvii, 382.

<sup>4</sup>Burget: *Am. Jour. Physiol.*, 1917, xlv, 492.

<sup>5</sup>Marine, Rogoff, and Stewart: *Ibid.*, 1918, xlv, 268.

—R. G. P.

### *Volunteer Medical Service Corps*

FOR the purpose of completing the mobilization of the entire medical and surgical resources of the country, the Council of National Defense has authorized and directed the organization of a "Volunteer Medical Service Corps," which is aimed to enlist in the general war-winning program all reputable physicians and surgeons who are not eligible to membership in the Medical Officers' Reserve Corps.

It has been recognized always that the medical profession is made up of men whose patriotism is unquestioned and who are eager to serve their country in every way. Slight physical infirmities or the fact that one is beyond the age limit, fifty-five years, or the fact that one is needed for essential public or institutional service, while precluding active work in camp or field or hospital in the war zone, should not prevent these patriotic physicians from close relation with government needs at this time.

It was in Philadelphia that the idea of such an organization was first put forward, Dr. William Duffield Robinson having initiated the movement resulting in the formation last summer of the Senior Military Medical Association with Dr. W. W. Keen as president—a society which now has 271 members.

Through the Committee on States Activities of the General Medical Board the matter of forming such a nationwide organization was taken up last October in Chicago at a meeting attended by delegates from forty-six states and the District of Columbia. This Committee, of which Dr. Edward Martin and Dr. John D. McLean—both Philadelphians—are respectively chairman and secretary, unanimously endorsed the project. A smaller committee, with Dr. Edward P. Davis, of Philadelphia, as chairman, was appointed to draft conditions of membership, the General Medical Board unanimously endorsed the Committee's report, the Executive Committee—including Surgeons-General Gorgas of the Army, Braisted of the Navy, and Blue of the Public Health Service—heartily approved and passed it to the Council of National Defense for final action, and the machinery of the new body has been started by the sending of a letter to the state and county committees urging interest and the enrollment of eligible physicians.

It is intended that this new corps shall be an instrument able directly to meet such civil and military needs as are not already provided for. The General Medical Board holds it as axiomatic that the health of the people at home must be maintained as efficiently as in times of peace. The medical service in hospitals, medical colleges and laboratories must be up to standard; the demands incident to examination of drafted soldiers, including the reclamation of men rejected because of comparatively slight physical defects; the need of conserving the health of the families and dependents of enlisted men and the preservation of sanitary conditions—all these needs must be fully met in time of war as in time of peace. They must be met in spite of the great and unusual depletion of medical talent due to the demands of field and hospital service.

In fact, and in view of the prospective losses in men with which every community is confronted, the General Medical Board believes that the needs at home should be even better met now than ever. The carrying of this double burden will fall heavily upon the physicians, but the medical fraternity is confident that it will acquit itself fully in this regard, its members accepting the tremendous responsibility in the highest spirit of patriotism. It will mean, doubtless, that much service must be gratuitous, but the medical men can be relied upon to do their share of giving freely, and it is certain that inability to pay a fee will never deny needy persons the attention required.

It is proposed that the services rendered by the Volunteer Medical Service Corps shall be in response to a request from the Surgeon-General of the Army, the Surgeon-General of the Navy, the Surgeon-General of the Public Health



Service, or other duly authorized departments or associations, the general administration of the corps to be vested in a Central Governing Board, which is to be a committee of the General Medical Board of the Council of National Defense. The State Committee of the Medical Section of the Council of National Defense constitutes the governing board in each state.

Conditions of membership are not onerous and are such as any qualified practitioner can readily meet. It is proposed that physicians intending to join shall apply by letter to the Secretary of the Central Governing Board, who will send the applicant a printed form, the filling out of which will permit ready classification according to training and experience. The name and data of applicants will be submitted to an Executive Committee of the State Governing Board, and the final acceptance to membership will be by the national governing body. An appropriate button or badge is to be adopted as official insignia.

The General Medical Board of the Council of National Defense is confident that there will be ready response from the physicians of the country. The Executive Committee of the General Medical Board comprises: Dr. Franklin Martin, Chairman; Dr. F. F. Simpson, Vice-chairman; Dr. William F. Snow, Secretary; Surgeon-General Gorgas, U. S. A.; Surgeon-General Braisted, U. S. Navy; Surgeon-General Rupert Blue, Public Health Service; Dr. Cary T. Grayson; Dr. Charles H. Mayo; Dr. Victor C. Vaughan; Dr. William H. Welch.

### *Immunity to Tuberculosis*

“ONLY a tuberculous animal is immune to tuberculosis.” This statement which would appear a paradox is yet true. It has been proved that one tubercle focus within an animal renders that animal immune to moderate reinfection from without. Should the focus be completely removed, this comparative immunity is also completely removed.

The animal with a focus of tuberculosis can in time, however, be overwhelmed by bacilli escaping from such an area should the encapsulation yield to destructive influence from within and at times probably to forces from without.

A safely encapsulated tuberculous focus is probably present in the bodies of most human beings. Such safe infection, the result of accident and of bodily resistance, protects the majority of mankind against active disease, yet Nature, in accomplishing such infection, and “vaccination” in the many, destroys a large number of our race. The evidence of autopsies corroborates what is learned through tuberculin tests that large numbers of people contain in their bodies such foci of tubercles. In possibly a majority of cases these foci are best found in lymph nodes, and often many of the tubercle bacilli found in these nodes prove to be dead.

Success in medical investigation is usually attained by imitating as closely as possible the processes of Nature. We triumph over diphtheria by adding to Nature's efforts quantities of antitoxin. We can easily conceive that Pasteur's employment of attenuated vaccines, the employment of dead bacteria so suc-

cessful in typhoid immunization, some of the accidents of bacterial dosage, may be now and then Nature's methods. Can we imitate Nature at her best in the production of a focus of tubercles in an animal, without the danger of following her at her worst—the destruction of the animal through tuberculosis?

A step in such a direction is possibly suggested in experiments, referred to by Krause<sup>1</sup> as simple and ingenious, a preliminary report of which has recently been published by Webb, Ryder, and Gilbert.<sup>2</sup> These workers have demonstrated that tuberculous lymph nodes can be transplanted into normal guinea pigs and that immunity, similar to that of a tuberculous animal will result. In time, however, such an immunized animal was found to succumb, through the escape of tubercle bacilli from within the transplanted focus. The attempts to provoke a stronger encapsulation of the lymph node by the employment of irritants also lacked success.

The results of surrounding these infected lymph glands with collodion, fish skin, etc., will be looked forward to with interest. Such "encapsulation" would probably allow immune processes resulting from the conflict of bacilli and lymphoid structures, to permeate into the host, yet should inhibit easily a spread of the bacilli. It will be important to determine the length of life of these bacilli, and also that of the tissue cells; these we understand are being investigated.

Heymans,<sup>3</sup> of Belgium, has reported several times that some immunity could be procured in animals by planting within them tubercle bacilli cultures which were enclosed in sacks made of the marrow membrane of the reed cane. Such a procedure of culture *in vivo* was first suggested by Metchnikoff. Heymans employed broth, exudates, and in some series, polymorphonuclear exudates for the culture media in these sacks. In general the bacilli were found to increase and multiply. Such sacks suspended in tubes of broth media were found to transmit tuberculin qualities to this media. Heymans reports in some experiments little if any immunity was produced in the animals receiving these sacks, and again in other experiments he reports some successes, not only in the production of some immunity to infection, but also as a useful procedure for the treatment of infected animals.

Djounskowsky is quoted as stating that toxins from the retained bacilli would diffuse through such an osmotic membrane. In diphtheria work a collodion membrane is employed to separate toxins from antitoxins, toxins diffusing through the membrane.

Jobling<sup>4</sup> has stated that unless a bacterial organism is tryptogenic a nourishment containing amino acids or peptones must be supplied. Such foodstuffs will pass through a collodion membrane.

That tubercle bacilli enclosed in collodion or other sacks will survive many months we know from the work of Heymans, who found virulent bacilli after eight months; and from the experiments of A. S. Griffith<sup>5</sup> of the British Royal Commission on Tuberculosis. This investigator attempted to change the human type of tubercle bacillus into the avian type by placing collodion capsules containing human tubercle bacilli within the peritoneal cavities of fowls and pigeons. The bacilli in some instances were found to be alive after more than two years.

Heymans' results suggest that tuberculin products were alone diffused, and

that the possible slight immunity demonstrated might be dependent on these. Products resulting from a conflict between bacilli and cells were not present.

It is recognized that in immunity to tuberculosis we are dealing with humoral as well as cellular qualities. The mechanism is probably very complex.

Much and Leschke<sup>6</sup> employed extracts of highly immunized animals and of tuberculous animals. These extracts were prepared from different organs and even from whole guinea pigs. Their conclusion was that the inoculation of such extracts failed to transmit any cellular immunity. Much, the advocate of partial antibodies in the search for humoral immunity, states that even with this determined no end is reached because cellular immunity must also be investigated.

It must be evident as the result of such vast experimentation in tuberculosis that the continued conflict of living bacillus and of living cell produces some product essential to the production of immunity.

The transplantation of a tuberculous lymph node or a piece of tuberculous liver is a new idea in experimental work for the production of immunity and opens up large possibilities for investigations.

Can the vital conflict, represented in such a transplant, be kept safely isolated without injury to the transplant and without injury to the host, a method of protection against tuberculosis will have been found.

The publication of this preliminary report will allow many laboratories to engage in this research.

#### BIBLIOGRAPHY

<sup>1</sup>Krause, A. K.: Editorial, *Am. Rev. Tuberc.*, February, 1918.

<sup>2</sup>Webb, G. B., Ryder, C. T., and Gilbert, G. B.: *Ibid.*

<sup>3</sup>Heymans, J. F.: *Arch. internat. de pharmacod. et de therap.*, 1905, xiv; *Semaine méd.*, 1907, p. 106; *Ibid.*, 1908, p. 119.

<sup>4</sup>Jobling: Personal.

<sup>5</sup>Griffith, A. S.: Royal Commission on Tuberculosis, Final Report, 1911.

<sup>6</sup>Much and Leschke: *Beitr. z. Klin. d. Tuberk.*, xx, No. 3.

—G. B. H.

### *Lymphocyte Elements and Tuberculosis*

A VAST amount of research work has been published which was undertaken to prove that the lymphocyte elements play a very important part in the defense of an organism against the bacillus of tuberculosis. As Krause<sup>1</sup> correctly states, nothing in this regard is conclusively proved, and we must grant that any evidence we have is possibly only circumstantial.

That any minute foreign substance may be taken to a lymph node or be taken up perhaps by a phagocyte we know, yet when we witness a lymph node arresting a tubercle bacillus or a polymorphonuclear leucocyte engulfing a gonococcus—with at times a resulting destruction of these elements and a triumph for the infecting organism—we believe that usually a battle has been fought.

Now, it is true that any tissue of the body may at times be attacked by the bacillus of tuberculosis and triumphantly defend itself—one tissue better than another—from such attack.

Much research work has proved that the formation of a tubercle in any

vulnerable tissue is a defensive mechanism of nature. Of what, then, is such a tubercle composed? It is composed of the giant cells, the so-called epithelioid cells, and the zone of lymphoid or mononuclear cells.

Whatever tissue, therefore, that defends itself against the bacillus of tuberculosis appears to receive the help of the lymphoid cells. Exudates from tuberculous processes also characteristically contain lymphocytes and the contents of cold abscesses suggest the triumphant bacilli may "enslave" the lymphocyte ferments. It is not necessary to inquire here into the origin of the giant and epithelioid cells.

The mysteries of natural processes are often difficult to interpret, yet many such processes are in time easily and reasonably explained. We can hardly conceive that Nature would send the lymphocyte cell into what we regard as a possible conflict unless she needed it there.

It is true that at times, especially in "immune" animals, a tubercle may appear to be formed and even absorbed without the presence of lymph cells being noted in corresponding specimens. Yet is it completely proved that not even a few are present possibly very early in the process? The qualities discovered in and attributed to the lymphocyte cell have been related here<sup>2</sup> before, so it is not necessary to repeat them. That immunity to tuberculosis is accompanied by a marked lymphocytosis, or that lowered resistance is contingent on lymphopenia are expectations possibly not proved, yet some investigations appear to decidedly support them.

That important immune and possibly specific processes are provided by the lymphocyte cells we must continue to suspect by virtue of the constant presence of such elements—possibly only as "infantry"—in the conflict of tissues and tubercle bacilli. The lymph nodes themselves must, therefore, also be judged as defensive as well as mechanical hindrances to the development of tuberculosis.

*"Natura non facit inutile aut superflua."*

#### BIBLIOGRAPHY

<sup>1</sup>Krause, A. K.: Am. Rev. Tuberc., Feb., 1918.

<sup>2</sup>Webb, G. B.: Immunity in Tuberculosis, Jour. Lab. and Clin. Medicine, 1916, i, 414.

—G. B. W.



# *The Journal of Laboratory and Clinical Medicine*

VOL. III.

ST. LOUIS, APRIL, 1918

NO. 7

## ORIGINAL ARTICLES

### AN INVESTIGATION OF CERTAIN PHENOMENA OF ALLERGY WITH SPECIAL REFERENCE TO THE RESPIRATORY AND CIRCULA- TORY SYSTEMS IN RELATION TO THE CAUSE OF DEATH\*

BY MORT D. PELZ AND D. E. JACKSON, PH.D., M.D., ST. LOUIS, MO.

THE cause of death in anaphylactic shock has been supposed to vary in different species of animals, and the associated phenomena have been held to be widely dissimilar. In the sensitized guinea pig a fatal bronchoconstriction is believed to be produced by the provocative injection of the protein, while in dogs most observers have considered the cause of death to be immediately associated with the vascular derangement and weakness. Bronchoconstriction has been thought to be almost, if not entirely, absent in these animals, and aside from the changes in the vasomotor apparatus, pathologic developments in the liver, intestines, heart, etc., have been noted by Richet,<sup>1</sup> Biedl and Kraus,<sup>2</sup> Pearce and Eisenbrey,<sup>3</sup> Edmunds,<sup>4</sup> Robinson and Auer,<sup>5</sup> Schultz,<sup>6</sup> Weil,<sup>7</sup> Manwaring,<sup>8</sup> Dale,<sup>9</sup> Simonds,<sup>10</sup> Voegtlin and Bernheim,<sup>11</sup> and others.

In the present series of experiments we have used dogs sensitized by three injections of the protein, injected subcutaneously at intervals of about five days. At each injection six to ten cubic centimeters of the protein were used, either normal horse serum† or egg albumen being given to produce the sensitization. After twenty-one or more days the animals were etherized, pithed, and prepared for recording carotid blood pressure. Then arrangements were made for recording the contractions or relaxations of the bronchioles by a method which was described in detail by one of us (D. E. J.)<sup>12</sup> some years ago. For this latter purpose the special piece of apparatus devised, when fitted into the

\*From the Department of Pharmacology of Washington University Medical School, St. Louis, Mo.

A preliminary report of this work was given before the American Pharmacological Society on Dec. 29, 1917, at Minneapolis, Minn.

†We have used normal horse serum with preservative sold by Parke, Davis & Co. Presumably the preservative is tricresol.

thorax through a median incision in the sternum, holds the chest wall rigidly distended, and keeps the chest cavity practically air-tight. The thoracic cavity is thus shut off from the external air except by communication through two tubes, one of which serves as an adjustable by-pass and permits air to enter the chest, while through the other, air is intermittently aspirated from the thorax. Inspiration occurs by air entering the lungs through the trachea during the forcible aspirations. Between aspirations the lungs collapse from their own elasticity (expiration) or by a contraction of the bronchioles. The pressure and rate of aspiration are kept constant and regular, about thirty per minute. The amount of air entering or leaving the lungs is recorded by means of a tambour connected with the side tube of the tracheal cannula, and writing upon a smoked drum. The extent of the down stroke of the writing lever indicates the relative amount of air entering the lungs in inspiration, the up stroke, the amount leaving in expiration. Contraction of the bronchioles decreases the amount of air entering and leaving the lungs, and thus the amplitude of the record on the drum is lessened. With the dilatation of the bronchioles the amplitude of the tracing is increased, because a relatively larger amount of air passes through the trachea at each inspiration or expiration.

Usually the brain and medulla were destroyed by pithing. For the provocative or final injection of protein quantities varying from eight up to seventeen cubic centimeters were quickly injected into the external jugular vein by means of a large syringe.

Fig. 1 shows the anaphylactic response produced immediately after the injection of the antigen. Together with the marked drop in blood pressure, there results an intense bronchoconstriction which is partially relaxed by the injection of one cubic centimeter of adrenaline (1:10000). This substance frequently saves the animals if injected *early*; that is, before the bronchial spasm has reached its greatest intensity. After the spasm is thoroughly developed, no drug so far tried will cause dilatation of the bronchioles. Forcibly dilating the lungs for several respiratory cycles, does not cause the lungs to remain relaxed when the original aspiration pressure is resumed, as would occur after almost any of the bronchoconstricting drugs. This shows how firmly the bronchioles are constricted. After the anaphylactic shock is well advanced and the asphyxia has become intense, adrenaline is no longer, or only feebly, active. We do not believe as Simonds has suggested that the decreased activity of adrenaline during the deepest part of the anaphylactic shock may be due to some specific change in the irritability of the vasomotor ganglia or medullary centers. We however, have supposed this decreased response to adrenaline to be due to the extreme grade of asphyxia present, and not to any specific action of the anaphylactic process, for practically the same bronchoconstricting phenomenon is seen after injections of a number of the opium alkaloids, and in the latter case adrenaline behaves similarly. This tracing also shows the failure of atropine to relieve the constriction of the bronchioles after the production of the shock. Several minutes later sodium nitrite was given intravenously to this animal, and it also failed to produce a relaxation.

Fig. 2 shows the action produced by injection of the protein into a sensitized dog which had received atropine just prior to the provocative injection. Enough

atropine ( $1\frac{1}{4}$  mg.) had been given to prevent electrical stimulation of the vagus nerve from slowing the heart; yet this did not prevent the anaphylactic phenomena. After partial recovery from the shock this dog received five cubic centimeters of codeine, and a typical normal response for this drug was ob-

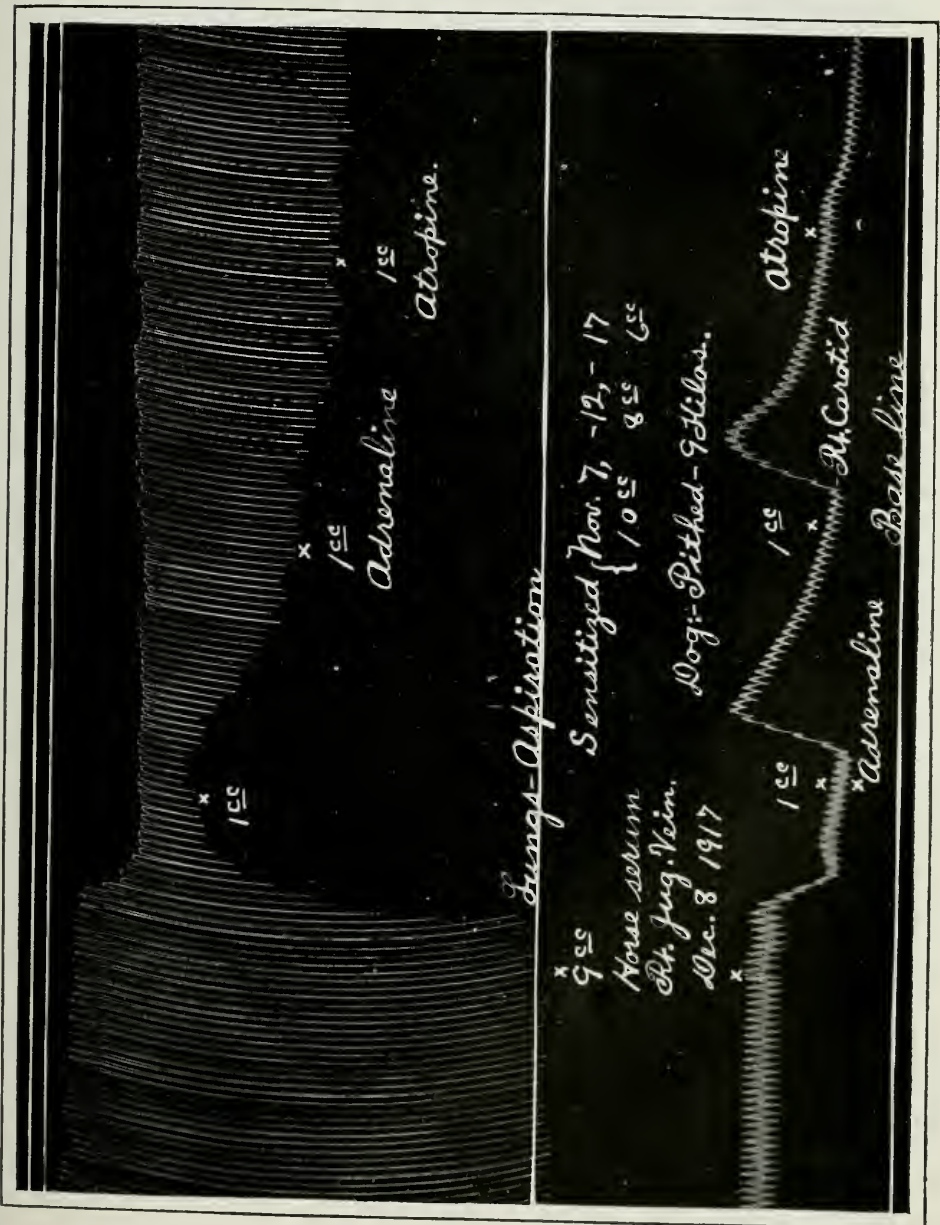


Fig. 1.—(For description, see text.)

tained (Fig. 3). As mentioned before, the action of codeine, heroine, etc., in dogs more closely resembles the anaphylactic phenomena than any substances with which we are acquainted. This similarity applies to both the bronchial



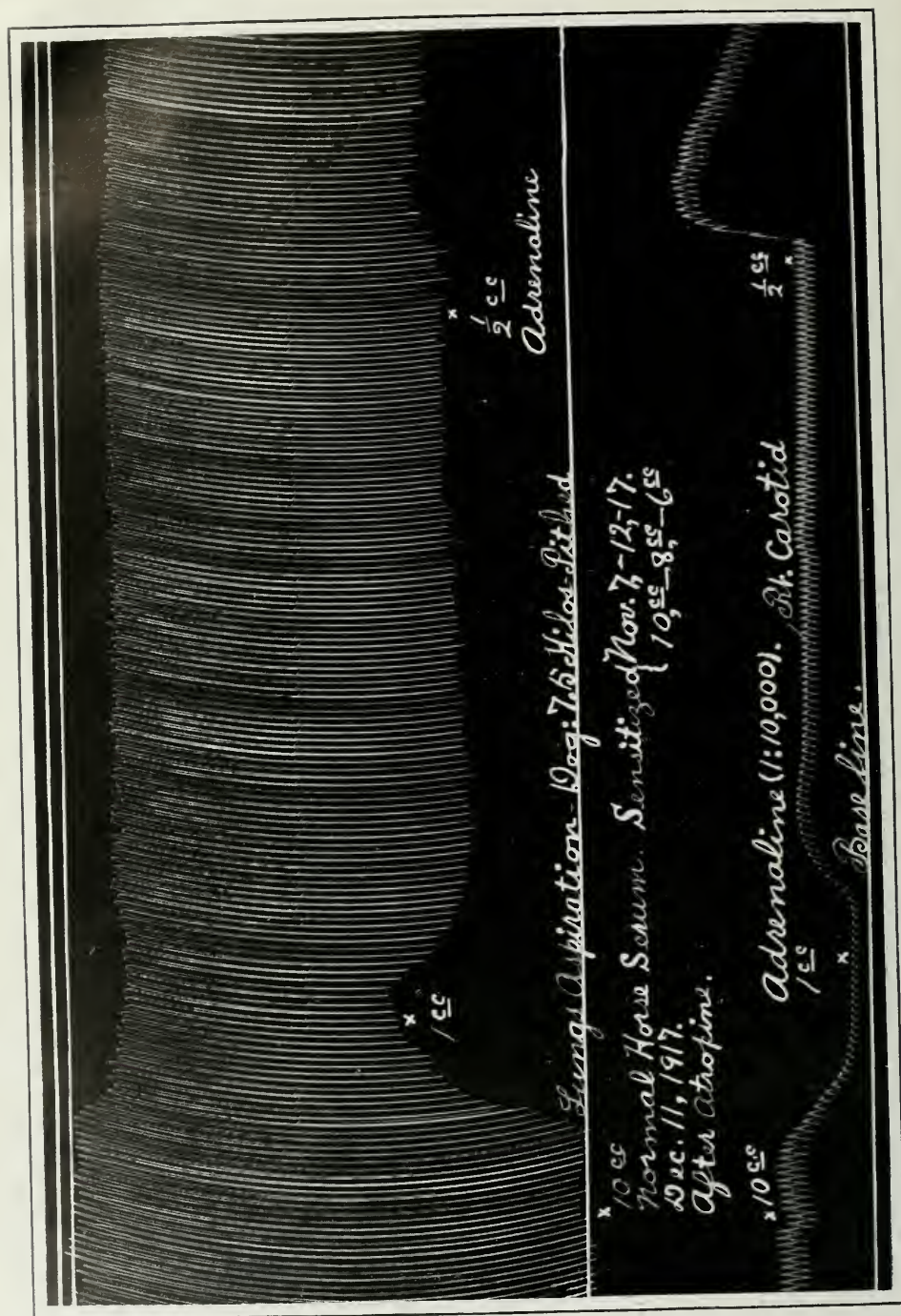


Fig. 2.

and blood pressure changes. Another point of similarity is that the action of the opium alkaloids in full doses is usually maximal, and after the animal recovers another typical response from the same substance can not be produced.



This resembles the maximal and single response produced by proteins injected into sensitized animals.

Again in the same dog after partial recovery ergamine (histamine) was given, which is shown to be active to practically its normal extent (Fig. 4). This drug has been thought by some observers to be similar (if not identical) to the substance liberated in the body during the anaphylactic shock and which produces the marked effects that occur. This is hardly probable when we consider that with a maximal dose anaphylaxis occurs but once, while the action of

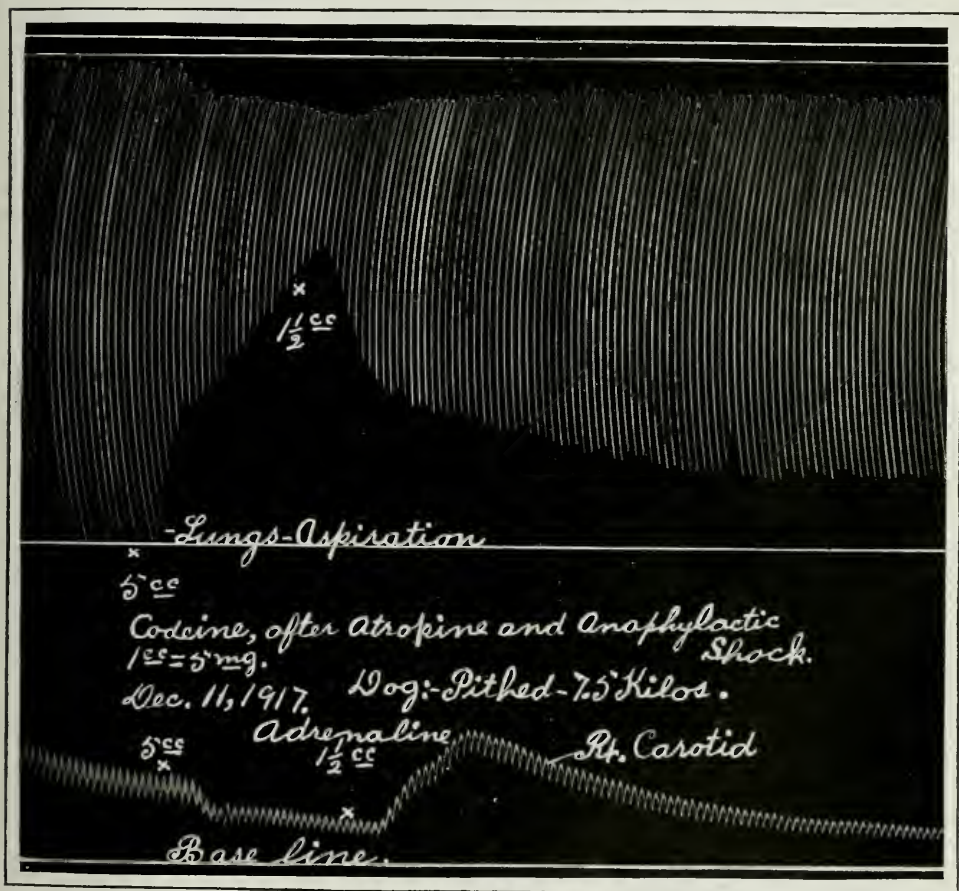


Fig. 3.

ergamine can be repeated several times, and even after partial recovery from the anaphylactic shock itself.

Fig. 5 shows the fatal bronchoconstriction in a well sensitized dog upon the injection of horse serum. Adrenaline apparently given fairly early after the production of the reaction could not cause recovery; due probably to the extreme grade of asphyxia already produced. The profound response in this animal was obtained after injections of pituitrin, and it is therefore apparent that there is no decreased susceptibility of a sensitized dog after injections of this substance.

In Fig. 6 is shown an especially marked response in an animal which was

sensitized to egg albumen. The bronchioles constricted so tightly and held on with such great tenacity that in a short time a fatal asphyxia was produced, and the production of bronchial relaxation by the administration of drugs was impossible.

The experiments of Manwaring,<sup>8</sup> Voegtlin and Bernheim,<sup>11</sup> Deneke,<sup>13</sup> and more recently of Richard Weil<sup>7</sup> have pointed towards the liver as essential to the vasomotor depressions and other changes which they have recognized as occurring in anaphylactic shock. In support of this view Weil found that the

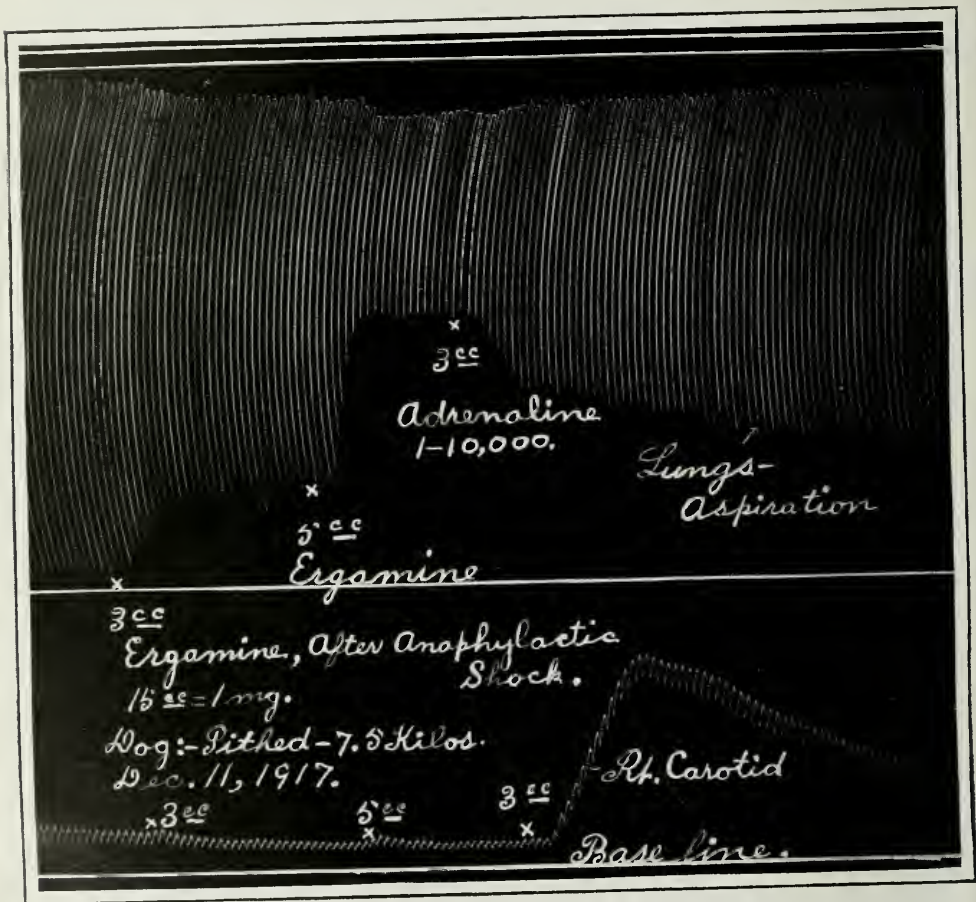


Fig. 4.

isolated liver of a sensitized dog when perfused with blood containing antigenic substance causes the blood to pass out of the organ with its coagulability either diminished or absent, while injection of antigen into one branch of the portal vein produced certain symptoms of shock and a profound congestion of that part of the liver supplied by the vein injected. Manwaring's results were obtained by means of side-tracking the portal blood by connecting the portal and external jugular veins and permitting the portal blood to flow directly to the heart, hirudin being used to prevent coagulation. Voegtlin and Bernheim made use of animals with an Eck fistula and combined this with ligation of the portal

vein near the hilus of the liver, the hepatic artery being temporarily clamped off. Both Manwaring, and Voegtlin and Bernheim in the animals which they so prepared, observed no symptoms of anaphylactic phenomena, and have accordingly considered the reactions of anaphylactic shock to be dependent on the liver, and not to occur without its activity.

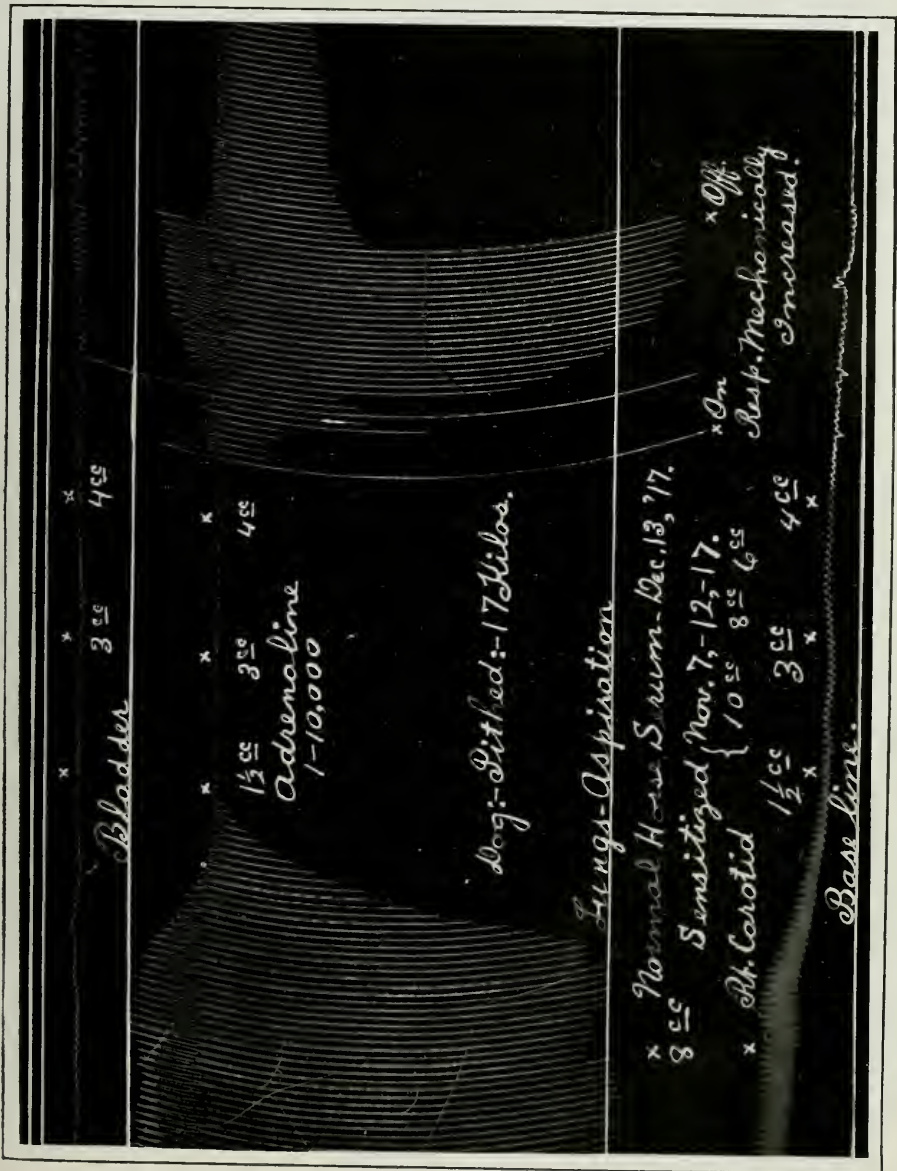


Fig. 5.

In our experiments we were able to obtain the typical anaphylactic reaction without the liver and also without the aid of any structures below the diaphragm. This was accomplished by clamping above the diaphragm the vessels carrying



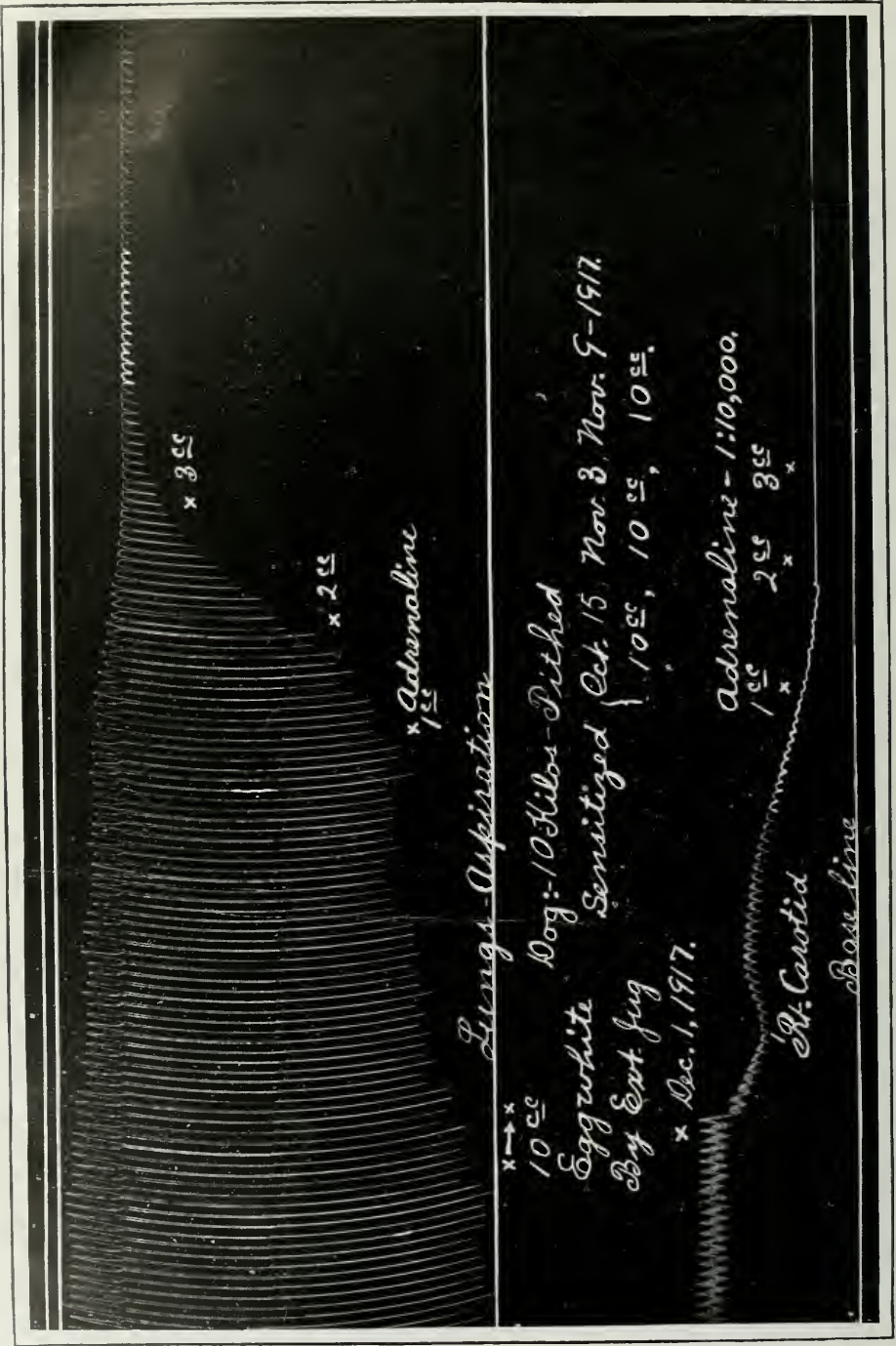


Fig. 6.

blood to and from the abdomen. The dogs were prepared as described above, and through the open chest was introduced a stout string threaded on two large needles. At a distance of about two centimeters laterally from each side of the



vertebral column and about five centimeters above the diaphragm the needles were pushed through intercostal spaces in the posterior chest wall to the exterior. They were then passed through holes in the dog board just beneath their point of exit from the chest. By drawing on the two ends of the string below the dog board and then clamping them, the aorta and azygos veins could be tightly

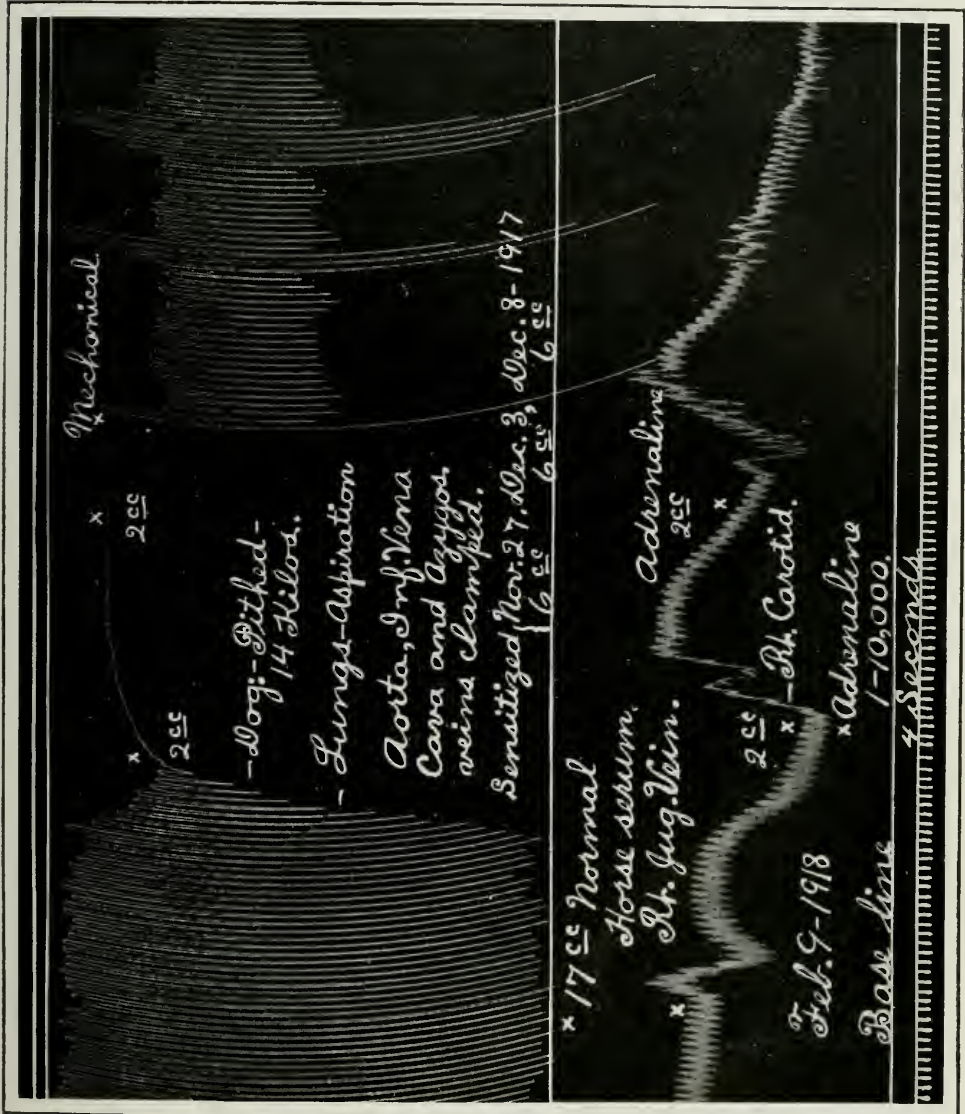


Fig. 7.

pressed against the vertebral column, and in this manner occluded. The inferior vena cava was ligated just above the diaphragm. The mammary arteries and veins were ligated when the chest was opened. Thus was circulatory communication with the abdomen completely stopped. This was verified by section of the femoral arteries. Then proceeding as in the experiments above described we in-

jected the antigen into the external jugular vein, and obtained a profound bronchoconstriction and a typical fall in blood pressure as usual (Fig. 7). Evidently the liver or intestines had nothing to do with these reactions which occurred exactly as in the intact animal.

In all of our experiments, in addition to obtaining the graphic record of the bronchial constriction we were able in looking through the window of the apparatus in the chest wall to observe the contraction and shrinkage of the lungs to about one-third their previous size, upon injection of the antigen. Apparently a very marked bronchoconstriction occurs in every case if the dog has been thoroughly sensitized. The criterion by which we have assumed the presence or absence of sensitization is the same as that used by preceding observers,<sup>3</sup> namely the fall in blood pressure.

We have as yet had no opportunity to study the split protein products of Vaughan,<sup>14</sup> which, as the splendid experimental work of Edmunds<sup>4</sup> has indicated, will at some future time, probably yield further interesting results along the lines indicated above.

#### CONCLUSIONS

1. From the experimental evidence at hand we may be certain that upon the injection of the antigen in highly sensitized spinal dogs, the asphyxia produced by the acute bronchial constriction may readily be the cause of death, and that the phenomenon occurs with the liver and other abdominal viscera wholly removed from the circulation.

2. The phenomena occur in those dogs sensitized to egg white equally as well as in those sensitized to normal horse serum.

3. The bronchial and blood pressure changes produced in acute anaphylactic shock bear a striking and almost complete resemblance to those produced by certain opium alkaloids, such as codeine, heroine, peronine, etc.

4. For producing relaxation of the bronchioles, and aiding in recovery in acute anaphylactic shock, adrenaline is the most dependable substance, but to produce the best effects the drug must be injected early, before the bronchoconstriction has had time to become too intense.

#### BIBLIOGRAPHY

- <sup>1</sup>Richet, C.: *Compt. rend. Soc. de biol.*, 1905, lviii, 112.
- <sup>2</sup>Biedl, A., and Kraus, R.: *Wien. klin. Wchnschr.*, 1909, xxii, 363; *Ztschr. f. Immunitätsforsch.*, 1909, vii, 205.
- <sup>3</sup>Pearce, R. M., and Eisenbrey, A. B.: *Jour. Infect. Dis.*, 1910, vii, 565; *Tr. Congress Am. Phys. and Surg.*, 1910, viii, 402; *Jour. Pharmacol. and Exper. Therap.*, 1912, iv, 21.
- <sup>4</sup>Edmunds, C. W.: *Ztschr. f. Immunitätsforsch.*, 1913, xvii, 105; *Ibid.*, 1914, xxii, 181.
- <sup>5</sup>Robinson, G. C., and Auer, J.: *Jour. Exper. Med.*, 1913, viii, 556.
- <sup>6</sup>Schultz, W. H.: *Jour. Pharmacol. and Exper. Therap.*, 1910, 1, 5; *Ibid.*, 1912-13, 3, 299.
- <sup>7</sup>Weil, R.: *Jour. Immunology*, 1917, ii, 525.
- <sup>8</sup>Manwaring, W. H.: *Bull. Johns Hopkins Hosp.*, 1910, xxi, 275.
- <sup>9</sup>Manwaring, W. H.: *Ztschr. f. Immunitätsforsch.*, 1910, viii, 1.
- <sup>10</sup>Dale, H. H.: *Jour. Pharmacol. and Exper. Therap.*, 1912, iii, 167.
- <sup>11</sup>Simonds, J. P.: *Jour. Infect. Dis.*, 1916, xix, 746.
- <sup>12</sup>Voegtlin, C., and Bernheim, B. M.: *Jour. Pharmacol. and Exper. Therap.*, 1911, ii, 507.
- <sup>13</sup>Jackson, D. E.: *Jour. Pharmacol. and Exper. Therap.*, 1914, v, 479.
- <sup>14</sup>Deneke, T.: *Ztschr. f. Immunitätsforsch.*, 1914, xx, 501.
- <sup>15</sup>Vaughan, V. C.: *Jour. Lab. and Clin. Med.*, 1915, i, 55; *Ibid.*, 1916, i, 630. (Herter Lectures for 1916.)

# STUDIES ON IMMUNITY WITH SPECIAL REFERENCE TO COMPLEMENT FIXATION\*

BY ALFRED BLUMBERG, SALT LAKE CITY, UTAH.

THE great assistance rendered by the complement-fixation method in establishing a correct diagnosis has made this test one of extraordinary importance. Its general use in the detection of various diseases, and in proper differential diagnosis, has led to its study by many investigators. Their reports have had a tendency to confirm the correctness of the Bordet-Gengou method, which was first applied by Bordet and Gengou in the investigation of the *B. typhosus*, the *B. coli communis*, the micrococcus intracellularis meningitidis, the *B. pertussis*, the *B. anthracis* and the *B. tuberculosis*. These tests were conducted to prove the correctness of the first findings, but the actual investigation of the possibilities of diagnosing pathologic conditions was conducted by Bordet, Gengou and others—Bordet-Gengou,<sup>1</sup> pertussis; Widal-LeSourd,<sup>2</sup> typhoid; Foix, mallein,<sup>3</sup> scarlatina; Schleissner, scarlatina.<sup>3</sup>

It is not important to cite a historical outline of further experimental work, in that the results are well known, not only in serologic laboratories, but also to the physician and veterinarian. Both professions appreciate the value of the test. One has but to look over the conditions—syphilis, gonorrhea, glanders, infectious abortion in cattle, pertussis, dourine, and tuberculosis—to understand the enthusiasm which the practical application of the modified complement-fixation method aroused. To these conditions we may add the test used for the identification of blood and meat-food in medicolegal cases. Although the manner of conducting the various tests is analogous, there is a difference in methods which enables the laboratory worker to draw his own conclusion as to the presence or absence of a certain disease. This difference lies exclusively in one of the reagents—the antigen.

In considering the antigen, it is not necessary to go into familiar theoretic details, but a few words about certain features interesting enough to be specially mentioned, are not out of place. Syphilis, gonorrhea, glanders, infectious abortion in cattle, pertussis, dourine, and tuberculosis can be diagnosed by the use of a different antigen for each disease. There are three specific groups of antigens:

*Group A* is composed of antigens which contain the specific organisms of a certain disease. These organisms are emulsified in saline, or the autolysate of the organisms is used.

*Group B* consists of an antigen which is essentially the liquid culture of a specific organism, and

*Group C* contains antigens which are the watery or alcoholic extracts of tissues.

In the diagnosis of gonorrhea, pertussis, glanders, infectious abortion in

\*The author wishes to express his appreciation to Marcus Ward Lyon, Jr., Ph.D., M.D., Professor of Bacteriology and Pathology, George Washington University, Washington, D. C., for suggestions and especially for the arrangement of the zoological nomenclature.



cattle, and dourine, antigens belonging to the first group are used. It is very interesting to note that these conditions are accompanied by a polymorphonuclear leucocytosis, or at least by a local pus condition. Dourine, in which there may be a lymphocytosis present, is, perhaps, an exception. Since the antigens already contain the etiologic factor of the respective diseases, they are specific antigens. Certain authors are inclined to consider the possibility of conducting successful complement-fixation by emulsifying the tubercle bacilli, and using the emulsion as an antigen in the detection of tuberculosis. Claims as to the results obtained are surprisingly optimistic, but can not be confirmed in certain instances. *A lymphocytosis is usually present in tuberculosis.*

There is but one antigen belonging to Group B in use, namely, the liquid egg medium tuberculosis culture of Besredka.<sup>4</sup> This antigen is considered to be specific, and will be referred to later in this study.

There is only one antigen of Group C deserving special mention—the antigen for syphilis, which was at first considered a true one, as it was prepared from an organ containing the etiologic factor of the disease. It was soon found, however, that it was the lipoid material, and not the treponema pallidum, which was responsible for the reaction. *Syphilis is accompanied by a lymphocytosis.*

Although complement fixations are classified in three groups, there is a fourth group—that of complement fixation without the presence of any antigen.

In considering a special antigen, the one for the complement fixation of tuberculosis has been selected. Some investigators make use of bacterial emulsions for antigenic purposes only, the foremost advocates in this direction being Zinsser and Miller,<sup>5</sup> who conducted their tests with an emulsion containing tubercle bacilli suspended in hypotonic salt solution. Eichhorn and Blumberg<sup>6</sup> could not substantiate the findings of Zinsser and Miller. The test conducted by Eichhorn and Blumberg dealt with sera derived from cattle; it was, therefore, necessary to conduct tests with sera of human origin. Altogether sixty sera were used for this purpose, the findings of which are found in Table I. The apparent objection was that the antigenic and the anticomplementary titers were too close. One other bacterial emulsion antigen has been in use in connection with comparative work conducted with the antigen of Zinsser and Miller. This bacterial emulsion (known as Friedmann's vaccine) contained the ichtyc type of the tubercle bacilli, but the results showed too many positives (See Table I). In establishing the diagnostic possibilities of a disease in which there is a lymphocytosis, these antigens were used to represent antigens of the first group, which were controlled by antigens of the second group. The latter antigen was that recommended by Besredka<sup>7</sup>—the liquid egg medium antigen, the preparation was the one modified by Eichhorn and Blumberg.<sup>6</sup>

It was found that the liquid culture when freed from the tubercle bacilli by means of Berkefeld filtration, did not present satisfactory antigenic properties. It is absolutely necessary to heat the culture before filtering it, but the heat applied is not enough to destroy any component properties of the antigen. It seems that the heat aids not only in the extraction of the organisms, but in the fine distribution of the fat. It must be remembered that since there is lipoid material present in the antigen, it may, in a luetic condition, have a tendency to give positive reactions. Bronfenbrenner<sup>8</sup> was perhaps one of the first in this



country to observe and study this phase of Besredka's antigen. There is, however, a possibility of overcoming a nonspecific reaction. The first step is to conduct a Wassermann test in every case where a positive reaction has been found; if the Wassermann test is also positive, the test for tuberculosis is repeated as before, except that three units of complement are used. With a few exceptions a modification of this type gives the desired results; negative reactions are obtained with nontuberculous sera derived from syphilitic individuals, and if positives are obtained in doubtful tuberculous cases, the reaction is considered positive for tuberculosis. This antigen has the advantage of being stable when ordinary precautions are taken. The following table gives the results obtained:

TABLE I

SUMMARY OF THE TESTS ON HUMAN SERA CONDUCTED WITH VARIOUS ANTIGENS FOR COMPARATIVE PURPOSE

CONDITION	ANTIGENS	XXXX	XXX	XX	X	-	TOTAL
1. Apparently healthy	Miller and Zinsser	3	1	2	4	16	26
	Friedmann	3	6	-	7	10	
	Liquid egg medium	-	2	-	2	22	
2. Clinical symptoms suspicious	Miller and Zinsser	2	1	1	1	4	9
	Friedmann	8	1	-	-	-	
	Liquid egg medium	4	1	1	-	3	
3. Clinical symptoms moderate	Miller and Zinsser	5	3	1	-	4	13
	Friedmann	11	1	1	-	-	
	Liquid egg medium	5	2	5	-	1	
4. Clinical symptoms progressive	Miller and Zinsser	3	-	2	-	2	7
	Friedmann	7	1	-	-	-	
	Liquid egg medium	4	-	1	-	2	
5. Clinical symptoms severe	Miller and Zinsser	-	-	-	1	4	5
	Friedmann	5	-	-	-	-	
	Liquid egg medium	-	1	-	-	4	
							60

Although Craig<sup>9</sup> reported impressive results, experiments were not conducted with his antigen, because in private communication,<sup>10</sup> he stated that the antigen prepared by him was so unstable that it was impossible to preserve it for more than a few hours, even though the utmost precautions were taken. To prepare a reagent identical to the one described by Craig was considered too difficult, and the experimental work in this direction had to be given up. Craig has recently adopted a modification of his antigen and published the results of his experiments on the subject.<sup>11</sup>

Other experiments were conducted in connection with those on complement fixation of tuberculosis, to determine the possibilities of diagnostic aids. Szaboky<sup>12</sup> stated that there were moments in the phase of the disease when agglutinations were demonstrable. Forty sera were tested with bacterial emulsions of the Zinsser-Miller and Friedmann types without noticeable reactions. In some instances the Friedmann vaccine presented a slight clumping, but of insufficient value to make it practical for diagnostic purposes.

The demonstration of precipitins was conducted with three different re-

agents: (1) The supernatant fluid of the Zinsser-Miller antigen, obtained by centrifugalizing the antigen; (2) the supernatant fluid of Friedmann's vaccine, obtained by centrifugalizing the vaccine; and (3) pseudoglobulins prepared from the serum of tuberculous cattle. The first two reagents were negative and consequently valueless, the practical value of the third is very limited. Forty sera were tested for precipitins; the Friedmann organism extract showed a fine ring not always of the same intensity.

The *conglutination test* was applied experimentally by Strengs<sup>13</sup> in the diagnosis for syphilis, and was first conducted in this country by Wehrbein<sup>14</sup> for the diagnosis of dourine. This method was used by the writer in detecting its practical value as related to tuberculosis. Altogether twelve sera were used for standardizing purposes; the practical value of this test in the diagnosis of tuberculosis was absolutely unsatisfactory. The accompanying tables show the methods of standardization:

TABLE II  
TITRATION OF ANTIGEN

NO. OF TUBE	SALT SOLUTION	COMPLEMENT	SERUM	ANTIGEN
1			T. B. serum 0.1	0.025
2			0.1	0.05
3	Sufficient	As	0.1	0.1
4			0.1	0.15
5	quantity	determined	0.1	0.2
6			0.1	0.25
7	to	by	0.1	0.3
8			0.1	0.35
9	make 1 c.c.	titration	0.1	0.4
10			0.1	—
11			Normal S. 0.1	0.10
12			0.1	0.20
13			0.1	0.30
14			0.1	0.40
15			0.1	—

After one hour at 37° C. add ox serum and emulsion of sheep blood.  
The result is read after three hours at 37° C.

TABLE III  
TITRATION OF OX SERUM

NO. OF TUBE	SALT SOLUTION	FRESH HORSE SERUM COMPLEMENT	INACTIVATED OX SERUM TITRATED 1:10	SHEEP EMULSION 5%
1	0.75	0.1	0.15	0.1
2	0.8	0.1	0.1	0.1
3	0.15	0.1	0.075 = 0.75	0.1
4	0.4	0.1	0.05 = 0.5	0.1
5	0.65	0.1	0.025 = 0.25	0.1
6	0.8	0.1	0.01 = 0.1	0.1
7	0.9	—	—	0.1
8	0.9	0.1	—	0.1
9	1.0	—	—	0.1

Three hours at 37° C.

Twice the smallest amount giving complete conglutination is used as titer.

In conducting complement fixations for diagnostic purposes with antigens of the third group (essentially of tissue extract origin), not only syphilis, but

tuberculosis and rabies were diseases towards which investigators looked with interest. Hammer<sup>15</sup> believed in the possibilities of an antigen derived from tuberculous organs in diagnosing tuberculosis, while Zell,<sup>16</sup> using the extracts of submaxillary and parotid glands of rabid dogs, believed that he could successfully demonstrate rabies by means of complement fixation. That Zell's assertion was incorrect has finally been settled; and since the studies of Schöning<sup>17</sup> concerning Zell's findings did not in any way substantiate his hopes, syphilis is the only disease in which tissue extracts can be applied for diagnostic purposes. There are many variations in the preparation of such extracts, those most widely used being the ones prepared from the guinea pig, or beef or human hearts. Some are common alcoholic extracts, some alcoholic extracts reinforced with cholesterol, and others are the acetone insoluble fractions of the alcoholic extracts.

There is much dispute as to the comparative values of the several antigens in conducting the test. Most of the disputes, however, turn around the non-specificity and inconsistency of the reactions in certain instances; true enough, the antigen is not specific insofar as no specific organisms are present, yet in

TABLE IV  
TITRATION OF COMPLEMENT

NO. OF TUBE	SALT SOLUTION	FRESH HORSE SERUM DILUTED 1:10	INACTIVATED OX SERUM	SHEEP BLOOD EMULSION 5%
1	0.8	0.1	two units	0.1
2	—	0.09 = 0.9	" "	0.1
3	0.1	0.08 = 0.8	" "	0.1
4	0.2	0.07 = 0.7	" "	0.1
5	0.3	0.06 = 0.6	" "	0.1
6	0.4	0.05 = 0.5	" "	0.1
7	0.5	0.04 = 0.4	" "	0.1
8	0.6	0.03 = 0.3	" "	0.1
9	0.7	0.02 = 0.2	" "	0.1
10	0.8	0.01 = 0.1	" "	0.1
11	0.9	0.1	—	0.1
12	0.9	—	two units	0.1
13	0.85	0.1	one unit	0.1
14	1.0	—	—	0.1

The result is read after three hours at 37° C.

The smallest amount of horse serum giving complete conglutination is the titer.

the hands of accurate workers, the test is of inestimable value. It is also true that there is a certain inconsistency in the test which expresses itself, not so much in excessively positive reactions, as in the reverse. Kaplan,<sup>18</sup> who believes in proving a negative reaction, states that, when conducting his tests, he added four units of amboceptor to the tube. There is no objection to this; as a matter of fact, the very first authors on the subject of complement fixation applied similar methods,<sup>19</sup> probably under the same impulse. Although such negative provocative tendencies may suggest the general occurrence of excessive false positive Wassermann reactions, this is not the case. Conditions often arise where the Wassermann test presents a negative reaction, even when the patient is known to have had syphilis and probably still has it. A more vigorous antigen was looked for as a remedy, and it was finally agreed that a cholesterol

reinforced antigen would meet the requirements. Today no test is performed without a cholesterolized antigen control. It must be added, however, that contradiction very often occurs concerning the reactions which result from the use of cholesterolized and noncholesterolized antigens. On many occasions positive tests are present with the cholesterolized antigen, where the noncholesterolized antigen presents negative results. Such a condition would cause no alarm if the cholesterolized antigen would present a specific and early reaction in suspicious cases, unfortunately no considerable number of early findings can

TABLE V

## MAMMALS

Ferruginous Tree-shrew, <i>Tupaia glis ferruginea</i> , Singapore.....	0.15...	0.20
European Hedgehog, <i>Erinaceus Europæus</i> .....	0.15...	0.10
Star-nosed Mole, <i>Condylura cristata</i> , Maryland.....	0.05...	0.05
Luzon Hairy-tailed Shrew, <i>Crocidura luzonensis</i> , Manila.....	0.05...	0.15
Murino Hairy-tailed Shrew, <i>Crocidura murina</i> , Baltistan.....	0.05...	0.20
Fish-eating Bat, <i>Noctilio leporinus</i> , South America.....	0.05...	0.40
Papuan Emballonurine Bat, <i>Emballonura nigrescens</i> , Papua.....	0.10...	0.20
Blood-sucking Bat, <i>Desmodus rotundus</i> , Ecuador.....	0.05...	0.50
Hoary Bat, <i>Nycterus cinereus</i> , New York.....	0.15...	0.6
Florida Free-tailed Bat, <i>Tadarida cynocephalus</i> , Florida.....	0.10...	0.40
Norway Rat, <i>Rattus norvegicus</i> , Manila.....	0.025.	0.15
Pocket Gopher, <i>Geomys bursarius</i> , Minnesota.....	0.05...	0.15

TABLE VI

## BIRDS

Tufted Puffin, <i>Lunda cirrbata</i> .....	0.12...	0.80
Kittiwake Gull, <i>Rissa</i> .....	0.15...	0.55
Pelican, <i>Pelecanus</i> .....	0.05...	0.40
Oyster-catcher, <i>Haematopus</i> .....	0.20...	0.80
Jacana, <i>Jacana</i> .....	0.15...	0.20
Goshawk, <i>Astur Palumbarius</i> .....	0.10...	0.20
Osprey, <i>Pandion</i> .....	0.025.	0.050
Yellow-headed Blackbird, <i>Xanthocephalus</i> .....	0.15...	0.40
Bobolink, <i>Dolichonyx oryzivorus</i> .....	0.25...	1.00
Waxwing, <i>Ampelis</i> .....	0.15...	0.20

TABLE VII

## REPTILES

Mons Island Ground Iguana, <i>Cyclura stejnegeri</i> , Mona Island, Porto Rico.....	0.10...	0.25
California Fence Lizard, <i>Sceloporus biserialatus</i> , California.....	0.05...	0.40
Boa Constrictor, <i>Boa Constrictor</i> , South America.....	0.20...	0.80
Hog-nosed Snake, <i>Lystrophis d'orbignyi</i> , Brazil.....	0.20...	0.20
Asiatic Snake, <i>Dinodon rufozonatum</i> , Eastern Asia.....	0.15...	0.30
Chicken Snake, <i>Elaphe confinis</i> , Wisconsin.....	0.15...	0.20
Band-tailed Chicken Snake, <i>Elaphe taeniurus</i> , Lorea.....	0.10...	0.20
Mud Turtle, <i>Cinosternon flavescens</i> , Texas.....	0.025.	0.50

be obtained with regularity, yet I have more than once obtained a positive test with a noncholesterolized antigen, and a negative one with a cholesterolized antigen using the serum of patients presenting primary lesions.

The study of the possibilities of testing tissue extracts obtained from sources



other than beef or guinea pig heart, or human liver or heart was of great interest. Thirty-seven varieties were tested for antigenic and anticomplementary doses; these specimens included extracts from 13 mammals, 10 birds, 8 reptiles, and 7 fishes. Tables V, VI, VII, and VIII will show the animals from which the extracts were obtained, and the results.

TABLE VIII

## FISHES

California Skate, <i>Raja bonoculata</i> , British Columbia.....	0.15...0.40
Cow Shark, <i>Notorhynchus maculatus</i> , San Francisco.....	0.15...0.20
Moray Eel, <i>Mursena nubila</i> , Asia.....	0.05...0.10
Cut-throat Trout, <i>Salmo mykiss</i> , Montana.....	0.20...0.80
Whitefish, <i>Leucichthys harengus</i> , Lake Superior.....	0.05...0.35
Common Carp, <i>Cyprinus carpio</i> , Texas.....	0.05...0.10
Great Club, <i>Leucisus lineatus</i> , Utah.....	0.05...0.15

That the values of the above findings are somewhat relative in character can not be denied, since there was no uniformity in the preparation of the extracts. Then, too, these extracts were not prepared for a special purpose; they were nothing more than the alcohol in which museum specimens of the above animals were preserved. One point, however, is of great importance; while there were a few extracts which had no antigenic value, there were some of decidedly valuable character. Whether or not the proportion tends to throw light upon the identities of cell characteristics in various animals can not, at present, be stated.

In connection with the complement fixation results, it is customary to speak of four, three, two, and one plus positives. Various attempts to apply methods for the determination of the strength of positive reactions were made, the most popular being that of centrifugalizing the blood corpuscles which remained unhemolyzed, and, by means of a scale system engraved on the centrifuge tube, determining the amount of corpuscles present, and the strength of the reaction. This method, while easily applied, is by no means faultless, and it seems that decreasing amounts of red blood corpuscles added to a set of tubes (containing the questionable serum, the antigen, the complement and the hemolysin) may serve to give more accurate results where they are desired. The following table demonstrates the method advised for the determination of the positive strength of a given serum.

If a serum does not hemolyze the amount present in the tenth tube, it is considered 100 per cent positive. The serum which hemolyzes Tube 10, but does not affect Tube 9, is considered 90 per cent positive; thus a serum hemolyzing to the fourth tube, No. 4 not being hemolyzed, however, is considered 40 per cent positive; the serum of the tube containing 0.9 c.c. blood emulsion, if incompletely hemolyzed, while the previous tube has been completely hemolyzed, is considered 10 per cent positive.

This titration method is of significance only in ascertaining the condition of a patient who has undergone treatment, and whose reaction was strongly positive before treatment.

TABLE IX  
TITRATION OF SERUM FOR COMPLEMENT BINDING POWER

NO. OF TUBE	SERUM	ANTIGEN	COMPLEMENT 5% SOLUTION	SALINE	INCUBATION TIME	HEMOLYSIN 2 UNITS IN 1 C.C.	SHEEP BLOOD EMULSION 5%	INCUBATION TIME
1	0.2	As determined by	1 c.c. to each	Sufficient quantity	1 hour at 37° C.		1.0	1 hour at 37° C.
2	0.2	titration	tube	to make			0.9	
3	0.2			3 c.c.			0.8	
4	0.2					Added to	0.7	
5	0.2					each tube	0.6	
6	0.2					after incubation	0.5	
7	0.2					time	0.4	
8	0.2						0.3	
9	0.2						0.2	
10	0.2						0.1	

#### THE COMPLEMENT FIXATION WITHOUT ANY ANTIGEN

Deluca<sup>20</sup> stated that in setting up a hemolytic system, when the urine of a pregnant was added, he succeeded in obtaining hemolysis, but in the presence of normal urine, the same system would not show hemolysis. The diagnosis of pregnancy by laboratory methods was very welcome after Abderhalden<sup>21</sup> had published his method. There was, of course, a great difference in the bases of his work, and that conducted in the present study. Abderhalden based his work on the specific enzymes present in the blood under certain conditions, and the demonstration of the enzymes was the essential basis of his method. Unfortunately many things rendered Abderhalden's test unpopular, and anything of more certain characteristics, and simple technic would be received with great appreciation by the clinician. Two hundred fifty-nine samples of urine were tested by the method here described, the results being so encouraging that the continuation of the study of the test seems to be of high importance.

The technic of the method is as follows: to 10 test tubes containing increasing amounts of urine, one-half c.c. of 20 per cent complement, 0.1 c.c. of 10 per cent sheep-blood emulsion and two units of hemolysin are added. The amount of urine contained in the first tube presenting complete hemolysis is the titer. The values found by the titration of urines are nearly absolute, in that once standardized to a certain hemolytic system, the titer of urines is constant.

To conduct the test, three tubes are set up for each specimen to be tested. The tubes are size No. 1, as used in the Noguchi test. The first tube contains the complete hemolytic system and 0.15 c.c. of the urine; the second contains the complete hemolytic system, and 0.25 c.c. of urine, while the third contains 0.25 c.c. of the urine, and the hemolytic system without complement. The last tube is the control tube. It is not necessary to have a second control tube which contains complement but no amboceptor; the absence of amboceptor does not, in many instances, prevent effective hemolysis. To each tube, 1 c.c. of physiologic saline is added, after which the tubes are well shaken and the test put in the incubator at 37° C. In case no hemolysis results within one hour, the test is considered negative for pregnancy, but if hemolysis occurs, the test is positive, the latter reaction usually setting in within the first 25 to 30 minutes. In some

instances instantaneous hemolysis occurs. If there is hemolysis present in the third tube, it is due to some cause other than pregnancy. There is no difficulty in reading the test, because unless the hemolysis is complete, there is no hemolysis present. The absence of hemolysis denotes absence of pregnancy; this point must be emphasized, in that so far I have experienced no test in which the urine of a pregnant did not present hemolysis.

Although the presence of hemolysis usually means pregnancy, there are certain exceptions (see tables below) which are, as a rule, of a type such that differential diagnosis is not unpractical.

TITRATION OF NORMAL URINE FOR INHIBITING PROPERTIES: AND TITRATION OF URINE OF A PREGNANT FOR HEMOLYTIC PROPERTIES

The accompanying tables demonstrate the method of titration for the inhibiting and hemolytic properties:

TABLE X

## TITRATION OF PREGNANCY URINE

NO. TUBE	URINE	COMPLEMENT	HEMOLYSIS	10% R. B. C.	SALINE	RESULT
1	0.05	One-half c.c.	0.1 c.c. rep-	0.1 c.c.	1 c.c.	Com. hemolysis
2	0.10	representing	resenting	for each	saline	Com. "
3	0.15	2 units to	2 units to	tube	to each	Com. "
4	0.20	each tube	each tube		tube	Com. "
5	0.25					Com. "
6	0.30					Incomplete
7	0.35					No hemolysis

TABLE XI

## TITRATION OF NORMAL URINE

NO. TUBE	URINE	COMPLEMENT	HEMOLYSIS	10% R. B. C.	SALINE	RESULT
1	0.05	One-half c.c.	0.1 c.c. rep-	0.1 c.c.	1 c.c.	No hemolysis
2	0.10	representing	resenting	for each	saline	" "
3	0.15	2 units to	2 units to	tube	to each	" "
4	0.20	each tube	each tube		tube	" "
5	0.25					" "
6	0.30					" "
7	0.35					" "

A few cases may illustrate the possibilities of applying the complement-fixation test for diagnostic purposes.

*Case No. 1.*—Mrs. H., last menstruation, Dec. 25, 1916; test performed Jan. 30, 1917, reaction positive. Symptoms of pregnancy set in two weeks later.

*Case No. 2.*—Mrs. W. C., age thirty-eight, last period Oct. 28, suspected change in life; called on physician Jan. 17, 1917. Reaction positive. Found to be gravid on examination.

*Case No. 3.*—Patient thirty-eight years of age; suspected change of life; no children before. Last menstruation four weeks previous; called on physician, reaction positive. Found to be pregnant three weeks later.

*Case No. 4.*—Mrs. K., two months since last menstruation, reaction positive. On operation found to be gravid about two months.

*Case No. 5.*—Patient twenty-eight years old, appendicitis, reaction positive, on operation found to be pregnant two months.

*Case No. 6.*—Girl, diagnosed to be pregnant, denied condition or possibilities, changed physician. Reaction negative. Examination negative. Diagnosed later, tuberculosis of mesentery.

These cases were selected for demonstration, a summary of the findings being given in Table XII.

TABLE XII

CONDITION	POSITIVE REACTION	NEGATIVE REACTION	TOTAL
Normal men	—	37	37
Girl child	—	1	1
Normal women of proper age	—	12	12
Women above age	—	2	2
Pregnancy present			
Condition first month	14	—	14
" second	8	—	8
" third	6	—	6
" fourth	2	—	2
" fifth	7	—	7
" sixth	7	—	7
" seventh	7	—	7
" eighth	5	—	5
" ninth	10	—	10
Pregnancy, no history of time	6	—	6
After fourth day of delivery	—	4	4
After ectopic pregnancy over 8 days	—	1	1
Pregnancy and albuminuria	1	—	1
Pregnancy and other pathologic condition	2	—	2
Cast in urine no albumin present	1	—	1
Albumin in urine no casts present	1	—	1
Albumin and casts in urine	1	—	1
Pus in urine	8	—	8
Sugar in urine	—	4	4
Hematuria	—	2	2
Appendicitis	1	27	28
Tuboovarian abscess	—	2	2
Pyosalpinx	—	2	2
Pyelonephritis	1	—	1
Vaginal abscess	—	1	1
Epidemic meningitis	—	1	1
La grippe	—	1	1
Measles	2	—	2
Pneumonia	—	1	1
Syphilis secondary	—	3	3
Tubes	—	2	2
Tuberculosis	1	5	6
Variola	1	—	1
Cerebral hemorrhage	—	1	1
Before chololithotomy	—	2	2
Before gastroenterostomy	—	1	1
Before hysterectomy	—	1	1
Gastric ulcer	1	2	3
Stenosis of the pylorus	1	—	1
Cardiac insufficiency	2	1	3
Mucus gastritis	—	1	1
Phlebitis	—	1	1
Varicose veins	—	1	1
Hemorrhoids	—	1	1
Colloid goiter	—	2	2
Myxedema	1	—	1



TABLE XII.—CONT'D

CONDITION	POSITIVE REACTION	NEGATIVE REACTION	TOTAL
Adenoma	—	1	1
Myoma	—	1	1
Carcinoma	—	1	1
Secondary anemia	—	1	1
Injuries	—	5	5
Chemicals taken:			
KI	—	2	2
Salvarsan	—	4	4
Men under ether	1	7	8
Cases without history	—	6	6
Old urines	1	1	2
Differential diagnosis between tumor and pregnancy and diagnosis found correct	—	2	2
Suspected pregnancy, diagnosis found to be correct later	22	8	30
After operation	1	5	6
Alcoholics	1	5	6
After removal of kidney of man	—	1	1
Edema of face	—	1	1

The question naturally arises, What is the mechanism of the test? Is it a true complement fixation, or is the change strictly of a chemical nature? As the investigations in this direction are not finished, no final answer can be given. The first step in proving that the change was not due to physical influences, was to determine the specific gravities of the various specimens. As a result it can be safely stated that specific gravity has scarcely any bearing on the test. With a specific gravity as high as 1.035 urines have given complete hemolysis, and urines with a specific gravity of 1.007 have failed to give results. Since the addition of excessive amboceptor would not cause changes in the reaction if no hemolysis were previously present, it seems probable that the test is that of a true complement fixation, but, on the other hand, if pregnancy is present, hemolysis will set in—perhaps with a slight delay—even if no amboceptor is added. The atypical reaction is demonstrable in urines of patients who suffer from some type of nephritis. The fact that conditions like smallpox, scarlatina or measles will present a condition which results in the hemolytic effect of the urine, while the urine itself is free of casts or albumen, is by no means a contradiction. These diseases may cause an initial lesion—perhaps temporary in type—which does not cause symptoms severe enough to produce albumin or casts, but is detectable by means of a seroreaction. As a matter of fact, in no case where there are casts present, will there be a lack of hemolysis, and in a few instances only will there be inhibitions presented where pus or albumin is found; in all these instances pus and albumin are due to a diseased kidney.

The above statistics tend to show that syncytial toxins present in the circulation of the gravid woman will affect the kidneys, the intensity of the nephritis depending upon the intensity of the toxin. Urines from such patients do not inhibit hemolysis, whether or not these urines are high in concentration.

The fact that pathologic conditions of other types do not present false tests, and that, unless the urine is old, no fixation of complement will set in where pregnancy is present, renders this test worthy of serious consideration.

## CONCLUSIONS

1. We have shown that true antigens (e.g., antigens which contain the etiologic factor of the disease, emulsified or autolyzed) will work only where there is a polymorphonuclear leucocytosis present.

2. While tuberculosis is a disease presenting a lymphocytosis, it will fix the complement with a specific antigen, which, however, is not a bacterial emulsion, but a heated egg medium culture containing small amounts of lipid.

3. Tissue extracts of mammals, birds, reptiles and fishes may serve as useful antigens for the diagnosis of syphilis.

4. If normal urine is added, the complement of the hemolytic system is affected without the presence of a specific antigen.

5. The presence of hemolysis in the test of the fourth group indicates either some type of affection of the kidney (even when no albumen or casts are demonstrable) or pregnancy, the two conditions frequently being separable by the clinical history of the patient.

6. The absence of hemolysis in a hemolytic system to which urine is added speaks against the condition of pregnancy.

7. In complement fixation without an antigen, reaction affecting the third tube (which serves as a control tube and should not hemolyze) speaks for nephritis, rather than pregnancy.

## BIBLIOGRAPHY

- <sup>1</sup>Bordet and Gengou: Sur l'existence des substances sensibilisatrices dans la plupart des serums antimicrobiens, *Ann. de l'Inst. Pasteur*, 1901, xv, 289.
- <sup>2</sup>Widal and Le Sourd: Recherches experimentales at clinique sur la sensibilisatrice dans le serum des typhiques, *Compt. rend. Soc. de biol.*, 1901.
- <sup>3</sup>Citron, J.: Die Methoden der Immundiagnostik und Immunotherapie und ihre praktische Verwertung, 1912.
- <sup>4</sup>Besredka, A.: Ueber die Fixationsreaction bei tuberculose der Meerschweinchen, Kaninchen und Menschen, *Ztschft. Immf.*, xxi.
- <sup>5</sup>Miller and Zinsser: A Method of Producing Antigen for Complement Fixation in Tuberculosis, *Jour. Immunology*, I., No. 2, p. 181.
- <sup>6</sup>Eichhorn and Blumberg: Diagnosis of Tuberculosis by Complement Fixation, With Special Reference to Bovine Tuberculosis, 1917, *Jour. Agr. Research*, viii, No. 1.
- <sup>7</sup>Besredka, A., and Jupille, F.: Le bouillon a l'oeuf, *Ann. de l'Inst. Pasteur*, 1913, xxvii, No. 11, p. 1009-1017.
- <sup>8</sup>Bronfenbrenner and Rockman: A Note on the Use of Purified Antigen of Besredka, *Biochem. Bull.*, 1914, xi, pp. 375, 376.
- <sup>9</sup>Craig, C. F.: Observation upon Complement Fixation in the Diagnosis of Pulmonary Tuberculosis, *Am. Jour. Med. Sc.*, cl, No. 6, p. 781.
- <sup>10</sup>Private communication to Dr. A. Eichhorn.
- <sup>11</sup>Craig, C. F.: *Jour. Am. Med. Assn.*, March 10, 1917.
- <sup>12</sup>Szaboky, J.: Erfahrung über die praktische Verwertung der Complementbindung und andere Bacteriologischer und Serologischer Untersuchungen bei der Diagnose der Lungentuberculose, *Ztschr. Tuberk.*, xiv, No. 4, p. 249.
- <sup>13</sup>Strengs, D.: Die Conglutination und die Diagnose des Syphilis, *Beitr. z. path. Anat. u. z. allg. Path.*, 1911, li, 279.
- <sup>14</sup>Wehrlein, H.: Conglutination in the Diagnosis of Dourine (Trypanosomiasis of the Horse), *Jour. Infect. Dis.*, xvi, 451.
- <sup>15</sup>Hammer: Die Serodiagnose der Rindertuberculose, *Deutsch. Tierarztl. Wchnschr.*, 1912, xx, No. 39, p. 593.
- <sup>16</sup>Zell: *Jour. Vet. Med. Assn.*, 1915.
- <sup>17</sup>Dept. Agr., Bureau Animal Ind.
- <sup>18</sup>Kaplan: Serology of the nervous system, 1914.
- <sup>19</sup>Mulzer: Practische Anleitung zur Syphilis diagnose, Springer, 1912.
- <sup>20</sup>Deluca: A Biologic Test for Pregnancy, *Semana méd.*, Sept., 1916, xxxix; *Abstr.*, *Jour. Am. Med. Assn.*, Feb., 1917.
- <sup>21</sup>Alderhalden, J.: Abwehrfermente des tierischen Organisms, Springer, Berlin.

## MENINGITIS AT CAMP GREENE

BY PAUL G. WOOLLEY, CAPTAIN, M. O. R. C., CAMP GREENE, CHARLOTTE, N. C.

CEREBROSPINAL meningitis of the meningococcic type has been a matter of great concern in the training camps of the United States during the past winter. It is, therefore, useful to place on record such data regarding this disease as may be available in order that in future times more may be known of the epidemiologic occurrence of it, and that if possible the laws governing its spread may be worked out.

In Camp Greene the disease was at no time truly epidemic though in one organization at least it acquired something of the appearance of epidemicity. This will be evident from a glance at the table which shows that in a period of something over two months only thirty cases occurred in a camp population varying between 30,000 and 40,000. Such an incidence in such a population can not be called epidemic. To be strictly accurate there were but 29 cases, for one of the thirty (No. 21) was of the pneumococcic type as proved by bacteriologic examination. Under the conditions of camp life such as prevailed during the past winter, with exceedingly cold weather, with sleet and rain alternating, with the men practically living in the mud, wet from one day's end to another, huddling together for warmth,—in other words with all the best conditions for the transmission of infection,—such an incidence can not be called epidemic. As a matter of fact it is a surprising fact that the sick rate was as low as it has been, and the amount of diseases that occurred from all causes speaks well for the care exercised over the men.

Cases of meningitis began to appear in December, 1917, and after the appearance of the first case of the disease continued to appear sporadically during January and February as shown in the table.

Certain facts relating to some of these cases are interesting and are to be borne in mind in deciding to what extent the camp conditions were responsible for the persistence of the disease. For instance,—

Case No. 28 had not been in camp for at least a month before he was relieved from duty with the Provost Guard in Charlotte, N. C., and reported back to his organization. Two days after returning to camp he was taken ill with meningitis.

Case No. 24 had, like the preceding one, been on duty with the Provost Guard in Charlotte N. C. Immediately upon his return to duty with his organization at Camp Greene he was placed in quarantine with a group of measles contacts and sent to the Detention Camp. After eight days in quarantine he developed meningitis. None of his tent or squad mates had the disease.

Case No. 14 was a recent recruit who had been separated from his battery with a group of other recruits with whom he was being trained. He had been assigned to his regiment about eight days when he developed meningitis. None of the other members of his group developed the disease.

CASE NO.	DATE REPORTED	CO. OR BATT.	ORGANIZATION NO.	REMARKS
1	Dec. 18, 1917.	B	9	
2	Dec. 21, 1917.	I	8	
3	Jan. 3, 1918.	G	8	
4	Jan. 3, 1918.	L	19	
5	Jan. 4, 1918.	E	12	
6	Jan. 9, 1918.	L	8	
7	Jan. 9, 1918.	K	12	
8	Jan. 9, 1918.	M. G.	19	
9	Jan. 9, 1918.	L	19	
10	Jan. 11, 1918.	H	1	
11	Jan. 11, 1918.	A	9	
12	Jan. 12, 1918.	D	10	
13	Jan. 12, 1918.	F	14	
14	Jan. 15, 1918.	E	15	Recruit.
15	Jan. 16, 1918.	33 Amb. Co.	26	
16	Jan. 17, 1918.	A	16	
17	Jan. 17, 1918.	A	15	Recruit.
18	Jan. 18, 1918.	E	3	
19	Jan. 20, 1918.	D	1	Developed in Detention Camp.
20	Jan. 20, 1918.	K	5	
21	Jan. 25, 1918.	C	9	Pneumococcus case.
22	Jan. 29, 1918.	Hdqs. Co.	19	
23	Feb. 1, 1918.	M. G.	6	
24	Feb. 4, 1918.	B	15	Charlotte case.
25	Feb. 8, 1918.	K	10	
26	Feb. 8, 1918.	A	7	
27	Feb. 10, 1918.	Hdqs. Co.	12	
28	Feb. 11, 1918.	F	14	Charlotte case.
29	Feb. 12, 1918.	D	6	
30	Feb. 13, 1918.	C	15	

Case 17 was also a recruit who immediately upon his arrival at camp came in contact with a case of measles and was promptly sent to the Detention Camp where he later developed meningitis.

In none of these cases, certainly, did the regimental conditions have anything to do with the appearance of the disease, and, also, since each man was kept isolated with a different group of associates in none of whom meningitis appeared, the cases themselves were not factors in the further occurrence of the disease. They were in other words sporadic cases which did not become centers about which other cases developed. No plausible explanation of the original infections in the individual cases can be offered other than the one that in each case the causative organisms were present and merely waiting for the proper decrease of resistance of the individual before invading the tissues. It is a surprising fact that under the necessary conditions of living there were not many more cases. For days at a time the weather and ground conditions were such that the men could leave their tents only to perform the absolutely necessary duties. Drill was impossible and nothing remained for them to do but lie around in their tents trying to keep warm and waiting for a propitious day when their bedding and clothes could be aired. The conditions were the same for officers and men and the disease did not confine itself to the men.

It will be noted that an organization in which three of the cases occurred lay adjacent to another organization in which a number of other cases appeared.



It will also be noted that between these two organizations are two (17 and 18) in which there were no cases. In other words there seemed to be no evidence of spread from one regiment to a neighboring one.

The features connected with meningitis in organization No. 19 are unique for this camp. In this regiment the incidence suggested epidemicity, and also in it one company had more than one case of meningitis,—the only instance of this in the camp. In no instance, however, was there more than one case in a squad. In this regiment three cases were from Company L, and one each in the Machine Gun Company and Headquarters Company. One of the cases which developed in Company L had been detailed to the regimental infirmary where he had come in direct contact with the man from the Machine Gun Company who later developed the disease. This, so far as can be determined, is the only instance in Camp Greene in which immediate contact could be strongly suspected of being a factor in spreading the disease.

The only available statistics at hand indicate that there have been between 5 and 7 per cent of carriers in Camp Greene. These figures came from the

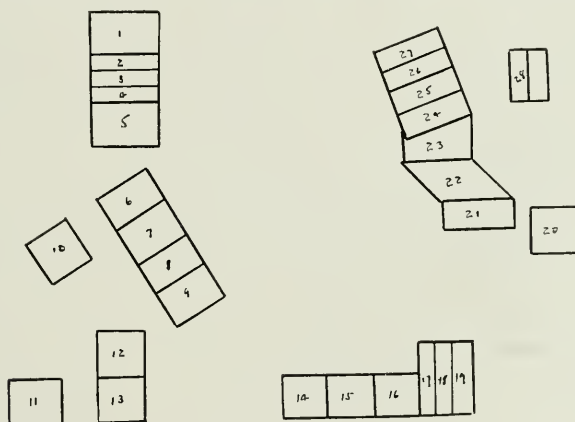


Chart to show the location of the different organizations in Camp Greene.

Detention Camp and are therefore too high, in all probability, for in this camp all meningitis carriers were isolated pending bacteriologic examination. It would appear therefore that the percentage of carriers in Camp Greene was smaller than in other camps. However that may be, it represents after all merely a guess because the only organizations which were cultured were those in which meningitis appeared and probably represents not more than half the personnel of the whole camp.

More interesting is the fact that in the only organization which made use of systematic nasal sprays since the first of the year not a single case developed, and also that in those organizations in which sprays were resorted to after the appearance of the disease no other cases appeared. This may be merely coincidental, but when one discovers that also following the adoption of sprays the total sick rate falls, especially that due to respiratory diseases, and bears in mind the current conception that the meningococci inhabit the nasal passages, one comes to have a very healthy respect for dichloramine-T as an agent for the prevention of diseases of upper respiratory tract origin. The organization num-

bered 7 in the chart has had the lowest measles and pneumonia rate in Camp Greene and is the only one which has systematically used the nasal spray. Its record is striking and forms a reasonable basis upon which to recommend that the routine use of nasal spraying with dichloramine-T be introduced into the camps for the prophylaxis of respiratory diseases.

## THE INTRAVENOUS USE OF RED MERCURIC IODIDE

BY L. W. ROWE, M.S., DETROIT, MICH.

THE value of mercury in combating spirochetal diseases, particularly syphilis, has long been recognized, but its exhibition in the body in a satisfactory form is attended by many difficulties. The insolubility of many of the salts of mercury coupled with their toxic action upon body cells has rendered them of very little therapeutic value. The bichloride of mercury because of its comparatively greater solubility and consequent effectiveness has been used wherever possible, but its very corrosive action upon body tissues when administered hypodermically, or even upon mucous membranes when applied in a more concentrated solution, have greatly limited its use. In combination with blood serum the bichloride has been used intraspinally with some success, but the amount of bichloride which combines with the serum is not very great and a large dose is therefore necessary. The intravenous use of a satisfactory solution of the bichloride is impossible because of the great danger from embolism due to coagulation of some of the proteins of the blood.

The red iodide of mercury,  $\text{HgI}_2$  (sometimes called biniodide) is as nearly insoluble in water as any of the salts of mercury, and for that reason is of no therapeutic importance. When combined with an equal amount of potassium iodide, a soluble compound is formed which is a very effective germicide, indicating that the characteristic action of mercury is exhibited. The efficiency of bichloride as a germicide is very great as is indicated by the fact that its germicidal coefficient is approximately one thousand (1000), or in other words that it is about one thousand times as efficient as pure carbolic acid, yet the average results, reported by various workers, of tests of solutions of mercuric iodide in potassium iodide show that this combination is fully five times as efficient as the bichloride, or 5000 times as efficient as pure carbolic acid. With this fact in mind it was thought advisable to attempt to obtain some idea concerning the possibilities of the use of mercuric iodide intravenously by determining the toxicity of this compound to animals when administered intravenously.

A search of the literature shows that Stassano and Gompel<sup>1</sup> have published several short articles in which they have commented upon the great germicidal activity and comparatively low toxicity of mercuric iodide and potassium iodide, but their experimental data has been largely concerned with the germicidal action and no definite comparisons could be made because of the lack of toxicity data.

<sup>1</sup>From the Research Laboratory, Parke, Davis & Co., Detroit, Mich.

<sup>2</sup>Stassano and Gompel: *Compt. rend. Soc. de biol.*, 1913, lxxv, 42-44; *Ibid.*, 1914, lxxvii, 9-11; *Compt. rend. Acad. d. Sc.*, 1914, clviii, 1716-19.

Lydston<sup>2</sup> in a very short report of some clinical results states that he has frequently administered red mercuric iodide dissolved in potassium iodide intravenously with no harmful effects.

The tests shown in the accompanying tables were carried out upon guinea pigs, dogs, and rabbits, in order to show the toxicity of red mercuric iodide and potassium iodide when given intravenously to animals. Two comparative tests of mercuric chloride were also made.

TABLE I

TOXICITY OF RED MERCURIC IODIDE DISSOLVED IN POTASSIUM IODIDE ADMINISTERED INTRAVENOUSLY TO DOGS

DOG NO.	WEIGHT IN KG.	DOSE IN GM. PER KG.	DIL.	TOTAL DOSE IN C.C.	RESULT	REMARKS
1	8	.015	1:100	12	Died	Dead in 2 hours
2	6.5	.010	1:20	1.3	Died	Dead in 20 hours
3	7.5	.008	1:20	1.2	Died	Dead in 2 days
4	9.5	.005	1:30	1.43	Died	Dead in 5 days
5	7.	.004	1:50	1.40	Died	Dead in 2½ days
6	9.	.004	1:20	.72	Died	Dead in 5 days
7	9	.0035	1:20	.63	Lived	Lived 3 weeks +
8	10.5	.0030	1:20	.63	Lived	Lived 3 weeks +
9	11.	.0025	1:20	.55	Lived	Lived 3 weeks +

M. L. D. .0040 gm. per kg. wt. of dog.

TABLE II

TOXICITY OF RED MERCURIC IODIDE DISSOLVED IN POTASSIUM IODIDE ADMINISTERED INTRAVENOUSLY TO GUINEA PIGS

NO.	WEIGHT IN GM.	DOSE IN GM PER KG.	DIL.	DOSE IN C.C.	RESULT	REMARKS
1	375	.008	1:100	.30	Died	Died during night
2	395	.005		.20	Died	Died during night
3	477	.004	1:1000	1.91	Died	
4	325	.004	1:500	.65	Died	
5	315	.004		.63	Died	
6	405	.004		.81	Died	
7	359	.004		.72	Died	
8	395	.0035		.70	Died	
9	458	.0035		.80	Died	
10	478	.0035		.83	Died	
11	455	.0035		.80	Died	
12	452	.0035		.79	Died	Died 6 days after dosing
13	445	.0030		.66	Died	
14	507	.0030		.76	Died	
15	519	.0030		.78	Died	
16	519	.0030		.78	Lived	Lived 10 days
17	436	.0030		.65	Died	
18	463	.0025		.58	Lived	Lived 10 days +
19	327	.0025	1:1000	.82	Lived	" " "
20	350	.0020		.70	Lived	" " "
21	488	.0020	1:500	.49	Lived	" " "
22	344	.0015	1:2000	1.00	Lived	" " "
23	375	.0010		.75	Lived	" " "
24	373	.0008	1:5000	1.50	Lived	" " "

M. L. D. is .0030 gm. per kg. body weight.

<sup>2</sup>Lydston, G. F.: Jour. Am. Med. Assn., 1916, lxxvii, 1446.

TABLE III

TOXICITY OF RED MERCURIC IODIDE DISSOLVED IN POTASSIUM IODIDE ADMINISTERED INTRAVENOUSLY TO RABBITS

NO.	WEIGHT IN GM.	DOSE IN GM. PER KG.	DIL.	DOSE IN C.C.	RESULT	REMARKS
1	2000	.025	1:100	5.0	Died	Dead in 1½ hours
2	1675	.015		2.51	Died	Dead in 3 days
3	1620	.010	1:50	.81	Died	Died during night
4	3150	.0080	1:100	2.52	Died	Dead in 1 week
5	1783	.0060	1:80	.86	Died	Dead in 1 week
6	2000	.0050	1:100	1.00	Lived	Lived 2 weeks +

TABLE IV

TOXICITY OF MERCURIC CHLORIDE ADMINISTERED INTRAVENOUSLY TO DOGS

NO.	WEIGHT IN KG.	DOSE IN GM. PER KG.	DIL.	DOSE IN C.C.	RESULT	REMARKS
1	6	.008	1:50	2.40	Died	Died in a few hours
2	7	6		2.10	Died	Died in a few hours
3	10	5		2.50	Died	Dead in 1 week
4	9	4		1.80	Lived	Lived two weeks
M. L. D. .005 gm. per kg.						

TABLE V

TOXICITY OF MERCURIC CHLORIDE ADMINISTERED INTRAVENOUSLY TO GUINEA PIGS

NO.	WEIGHT IN GM.	DOSE IN GM. PER KG.	DIL.	DOSE IN C.C.	RESULT	REMARKS
1	455	.008	1:200	.73	Died	Died during night
2	493	.007		.69	Died	Died during night
3	495	.006		.59	Died	Died during night
4	572	.005		.57	Died	Died during night
5	446	.004	1:400	.72	Died	Died during night
6	451	.003		.54	Died	Died within 2 days
7	286	.0025		.72	Died	Died 3 days later
8	252	.0025		.63	Died	Died 2 days later
9	286	.0020	1:1000	.57	Lived	Observed for 1 week
10	360	.0020		.72	Died	Died in 4 days
11	358	.0020		.72	Died	Died in 3 days
12	327	.0015		.49	Lived	Observed for 1 week
13	368	.0015		.55	Lived	Observed for 1 week
14	395	.0015		.59	Lived	Observed for 1 week
15	318	.0010		.32	Lived	Observed for 1 week
M. L. D. is .0020 gm. per kg.						

From data available in this laboratory the toxicity of phenol (carbolic acid) administered intravenously to guinea pigs is 0.2 gm. per kg. body weight. With this additional data Table VI of comparative toxicity and efficiency should be of value.

From Table VI the advantage of the use of either mercuric chloride or red mercuric iodide over the use of phenol, by comparison of toxicity and germicidal efficiency, can be readily seen. The figures in parenthesis indicate the comparison between mercuric chloride and mercuric iodide alone, using the figures obtained for mercuric chloride as unity. These figures show that although red mercuric iodide in potassium iodide when given intravenously is very little



TABLE VI

COMPARISON OF TOXICITY AND GERMICIDAL EFFICIENCY OF PHENOL, MERCURIC CHLORIDE, AND MERCURIC IODIDE

	GERMICIDAL COEFFICIENT	TOXICITY INTRAVENOUSLY TO GUINEA PIGS	TOXICITY INTRAVENOUSLY TO DOGS
Phenol	1	1	no data
Mercuric chloride	1000 (1)	100 (1)	.005 gm. per kg. (1)
Mercuric iodide in KI	5000 (5)	66% ( $\frac{2}{3}$ )	.004 gm. per kg. ( $1\frac{1}{4}$ )

if any more toxic (in the case of guinea pigs it is less toxic) than mercuric chloride, yet its germicidal efficiency is five times as great as that of mercuric chloride. It should consequently be greatly preferred to the bichloride if only because of its greater efficiency.

Another factor which is worthy of serious consideration is that in all the intravenous injections into animals of mercuric iodide (some of the injections being made rapidly) not one of the animals died suddenly following the injection. This would indicate that a solution of red mercuric iodide in potassium iodide has no very strong tendency to coagulate any of the constituents of the blood when introduced directly into the blood stream and thereby cause sudden death from embolism. On the other hand according to our observations, solutions of mercuric *chloride* have several times produced sudden death when administered intravenously to animals, indicating that embolism is often formed. This is very apt to happen if the injection is made rapidly.

Solutions of mercuric iodide like those of most mercury salts cause marked local irritation when administered subcutaneously or intramuscularly, so that the iodide will probably never become very popular for such methods of administration.

However, with the increase of our use of intravenous therapy because of advantages which it possesses in many instances and particularly because the most successful method of combating a severe systemic disease such as syphilis is by intravenous injection of such agents as salvarsan, etc., in solution, it seemed advisable to contribute the above tables of data to our knowledge of the pharmacologic action of such an important germicide as red mercuric iodide.

It is not the object of this paper to assure physicians of the safety of the intravenous use of this salt of mercury because this can not be assumed from animal experimentation alone. It is intended merely to point out certain therapeutic possibilities as indicated by animal tests. If these can be substantiated by carefully conducted clinical tests, as it is hoped they can be, the purpose of the article will have been attained.

## SUMMARY

Red mercuric iodide in combination with an equal amount of potassium iodide can be injected in solution into animals intravenously with comparative safety if reasonable care is exercised in the manner of injection and in the size of the dose injected.

It is very little if any more toxic than mercuric chloride, safer for intravenous use, and because of its greater germicidal efficiency should be found to be of therapeutic value.

## AN OUTLINE FOR THE COMBINED TEACHING OF PATHOLOGY AND BACTERIOLOGY IN SMALL MEDICAL COLLEGES\*

BY ELLIS KELLERT, M.D., ALBANY, N. Y.

AT no previous time in the history of American medical institutions has there been so great a demand for conservation of resources, concentration of effort, or emphasis of fundamental principles. The withdrawal of a large portion of the teaching and scientific staffs of all medical colleges for military service and the consequent reduction in the number of available assistants for teaching purposes calls for a rearrangement of courses and methods. This is particularly true of small medical colleges where the number of instructors barely suffice in normal times to carry on the work. For these reasons, and as the result of a successful experience of three years' duration, the following outline is offered as a suggestion in the conduct of classes in pathology and bacteriology.

It is needless to say that the student who is not thoroughly instructed in histology, physiology, and biochemistry has been poorly prepared for the study of pathology. The inability to visualize normal structures and functions makes it impossible to recognize and interpret abnormal changes, and, therefore, the essential conceptions of disease may be lacking. The structure of a lesion will have but little significance for the student not acquainted with histologic anatomy, and there must be developed in him the power to identify the cause and effect, and predict the remote result of the changes which he observes. With training in the recognition of isolated lesions will come the ability to conceive the general bodily changes. With these requirements in mind the instructor must plan to utilize his time and facilities to greatest advantage. The essentials are to be emphasized and the purely philosophic phases of the subject omitted. The didactic teaching should be reduced to a minimum and experimental demonstrations made as numerous as possible. The outline is also suggested at this time because many men without previous teaching experience have been suddenly called upon to conduct courses in pathology and bacteriology.

The tendency at present in medical schools is toward the "block" system of teaching the laboratory courses. The advantages of this system are that the student devotes himself entirely to the one subject which usually occupies half the school year. The teacher is free, therefore, for study or research during the remaining months. The chief disadvantages are the rapidity with which progress must be made and the possible lack of sufficient material during the brief period of the pathologic course. Great care must be exercised to avoid bewildering the student as the subject expands and the method here

---

\*From the Bender Hygienic Laboratory, Albany, N. Y.

suggested will accomplish much toward giving him a comprehensive view of the studies of pathology and bacteriology.

The work includes the following subjects: general and organal pathology, bacteriology, animal parasitology, tumors, and serology. The course is begun with exercises in bacteriologic technic. The classification of microorganisms is then studied, after which the student acquaints himself with the physiologic characteristics of bacteria. He then proceeds to the study of inflammation and the retrograde changes in tissues. The specific pathogenic bacteria are next investigated, first morphologically and culturally, immediately after which the lesions they induce are studied. There is thus given a complete picture of cause and effect and the association of a certain organism with a characteristic lesion is firmly fixed in the student's mind. Fresh tissues or preserved specimens from which microscopic sections have been made are exhibited and in his attempts at differential diagnosis, the student makes use of the freezing microtone or resorts to animal inoculation. By this method, fatigue from continuous concentration in one subject is avoided and interest is sustained by retaining the subject in its entirety. Thus by way of example, the bacteriology of the typhoid bacillus may be studied in the morning session and in the afternoon, the pathologic anatomy of typhoid fever. The teaching effort is reduced to a minimum, and the student gains a more comprehensive knowledge of the subject. After progress has been made, protocols of actual autopsies are read and discussed by the class in conference. The organs are exhibited, changes noted, the anatomic diagnoses tabulated and the lesions explained.

Although the above would seem to be sufficient to keep the student quite busy, yet he is found to have unoccupied time especially during the last month or six weeks when relieved of the extensive bacteriologic technic. Each student, therefore, is assigned a problem for investigation, which, except in the case of unusually capable students, should not be original, in order that a comparison may be made with the results of a previous investigation. If desired however, original problems may be undertaken and varied according to the mental capacity and technical ability of the individual student. Thus one man may be given the problem of determining the chief varieties of bacteria in the throats of twenty normal adults, another may attempt to isolate typhoid bacilli from the urine and stools, another will determine the antiseptic properties of certain chemicals, and still another study the bacteria found on hair brushes. Numberless other topics will suggest themselves. Pathologic studies involving animal inoculations may also be suggested, but such problems demand too much of the teacher's time and there is needless sacrifice of animal life. Experiments with the larger animals are best reserved for class demonstrations. The object of each study should be systematic work, minute observation, recording of data and careful technic. When the problem is finished, the paper is read before the class and discussed.

Lectures should be few, but demonstrations numerous. At the end of the half day's session a meeting of the class is held and the subject of the day discussed in conference. Great interest is evinced by students in going over an

autopsy protocol, for they soon learn that this form of study is stimulating; not only with reference to the work in hand, but also to related subjects. The method in reality is case teaching, and, when gross and microscopic material can be exhibited, is the ideal procedure.

In the outline which follows no headings are included for diseases of the nervous system\*, but these may be inserted at convenient points and new headings created such as diseases of the meninges, atrophic and degenerative sclerosis of the cord, of the brain-stem, cerebral hemorrhage, cerebral softening and others which will suggest themselves.

#### OUTLINE OF COURSE

Historical—classification.	Typhus.
Bacteriologic methods.	Poliomyelitis.
Staining.	Immunity.
Physiology of bacteria.	Phagocytosis.
Destruction of bacteria.	Opsonins.
Carbolic coefficient.	Agglutinins.
Blood—histological review.	Precipitins.
Theories of immunity.	Vaccines.
Inflammation.	Serums.
Pyogenic cocci.	Anaphylaxis.
Retrograde processes.	Serologic methods of diagnosis.
Streptococcus—pneumococcus group.	Tumors—
Gonococcus—meningococcus group.	Benign connective tissue tumors.
Pneumobacillus.	Malignant connective tissue tumors.
Pneumonia.	Endothelioma.
B. mallei.	Myoma.
Glanders.	Glioma.
Hemoglobinophilic bacteria.	Neuroma.
Whooping cough.	Benign epithelial tumors.
Influenza.	Malignant epithelial tumors.
B. pyocyaneus group.	Mixed tumors.
B. anthracis.	Teratoid tumors.
Anthrax.	Cysts.
Colon—typhoid group.	Animal parasites.
Dysentery.	Protozoa.
Typhoid fever.	Amebæ.
B. diphtheriæ.	Flagellates.
Diphtheria.	Ciliates.
Pseudo-diphtheria bacilli.	Amebic dysentery.
Acid-fast bacilli.	Sporozoa.
Tuberculosis.	Coccidia.
Leprosy.	Coccidiosis.
B. pestis.	Hemosporidia.
Plague.	Malaria.
The Anaerobic bacilli.	Sarcosporidia.
Anaerobic methods.	Vermes.
B. aerogenes capsulatus.	Trematodes.
B. of malignant edema.	Cestodes.
Gaseous gangrene.	Nematodes.
B. tetani.	Arthropoda.
Tetanus.	Ductless glands.
Pathogenic spirillæ.	Pernicious anaemia.
Cholera.	Leukemia.
Relapsing fever.	Hodgkin's disease.
Vincent's angina.	Spleen and lymph nodes.
Treponema pallidum.	Circulatory system.
Syphilis.	Lungs.

\*An extensive course in diseases of the nervous system was given by the department of neurology.



## OUTLINE OF COURSE—CONT'D.

The Higher forms of microorganisms.	Stomach and intestine.
Actinomycosis.	Pancreas.
Sporotrichosis.	Liver.
Thrush.	Genitourinary system.
Ringworm.	Female genitalia.
Favus.	Male genitalia.
Filterable viruses.	
Diseases of unknown etiology.	
Rabies.	

The outline simply indicates the general headings and should be amplified by the instructor by the addition of numerous subheadings. Because of present-day interest, such topics as measles, meningitis, pneumonia, gangrene, vaccination, and venereal diseases should be treated in great detail.

Owing to the abundance of surgical material in most laboratories, surgical pathology is prone to receive undue importance and the teaching may degenerate into the gross and microscopic examination of specimens, thus making the course a diagnostic one instead of a biologic study. This tendency is also present in clinical teaching laboratories in hospitals where the examination of blood and excreta receive undue consideration. Under such circumstances the student tends to become a technician and the intellectual study of medicine may be neglected. During the fourth year, a course in advanced pathology should be provided. In the light of his brief clinical experience, such study will be of great value to the student and should include a thorough consideration of functional pathology.

In most schools too little time is devoted to animal parasitology. This phase of comparative pathology is extremely interesting and instructive, elucidating principles of pathology not otherwise so readily attained. The lesions are pronounced, material abundant, and the injurious agents so easily procured that the subject should receive greater consideration. No other branch of pathology illustrates so well the relation between cause and effect. In fact, the study of animal parasites would at the present time, when such keen interest is manifested by the general public in hygiene and disease, be an exceedingly profitable subject for courses in biology. This plan has been suggested on a previous occasion by Dr. Theobald Smith and might well be adopted in premedical studies. The interest thus aroused in animal parasitology may result in the more active pursuit of comparative pathology.

The outline above presented is intended for small schools. There are those who feel that the subjects of pathology, bacteriology, and serology are sufficiently comprehensive to be conducted under separate departments. Where this is done, much overlapping and repetition occur and it is not advisable unless extensive resources, financial and otherwise, are available. Our experience has been that two instructors and one technician can readily conduct the above combined courses, omitting nothing considered necessary in teaching the principles of pathology and bacteriology. That changes in the methods of teaching these subjects are gradually coming about is indicated by such recent works on pathology by Mallory and also by MacCallum. It would be a serious error, indeed, to have the student regard these subjects in any other light than as one science.

# LABORATORY METHODS

## A CLINICAL METHOD FOR DETERMINING THE RESPIRATORY EXCHANGE IN MAN\*

BY R. G. PEARCE, B.A., M.D., CLEVELAND, OHIO

SINCE the determination of the respiratory exchange in man is of some importance in the study of certain diseases of respiration, circulation, and metabolism, and also because directions for carrying out the necessary procedures are not generally available, I have thought it might be of assistance to present here brief directions for the Tissot<sup>1</sup> and the Douglas methods.<sup>2</sup> These methods have been found to compare favorably in accuracy with others in use at present,<sup>3</sup> and because of their adaptability and simplicity they are specially suited for clinical work.

By these methods the energy metabolism of the body is calculated from

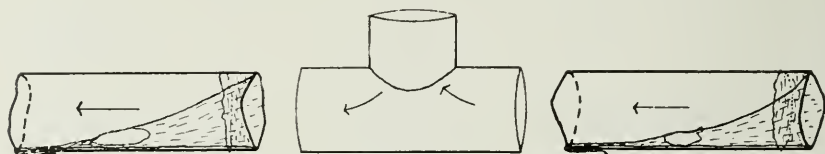


Fig. 1.—Valves used by the author.

oxygen consumption or carbon-dioxide excretion per minute (indirect calorimetry), the figures for which are determined from the minute volume and per centile gaseous composition of the expired air.

The subject breathes through valves which automatically partition the inspired and the expired air. The expiration from a number of respirations are collected in a spirometer or bag, and the volume of the respirations per minute is determined. The gaseous composition of the expired air is determined by gas analysis, and the oxygen consumption and energy output of the body are calculated from the data obtained.

### DESCRIPTION AND USE OF PARTS OF THE APPARATUS<sup>1</sup>

*The Mouthpiece and Valves.*†—The mouthpiece is made of soft pure gum rubber, and consists of an elliptical rubber flange having a hole in the center 2 cm. in diameter, to which on one side a short rubber tube is attached. On the opposite side of the hole, at right angles to the rubber flange, are attached two rubber lugs. The rubber flange is placed between the lips, and the lugs are

\*From the Cardiorespiratory Laboratory, Medical Service, The Lakeside Hospital, Cleveland, Ohio.

†The mouthpiece, nose clip, face mask, Douglas bag, and gas-analysis apparatus can be secured from H. N. Elmer, 1140 Monadnock Building, Chicago, Ill.

held by the teeth. The rubber tube of the mouthpiece is connected to the tube carrying the valves. The nose must be tightly closed if mouth-breathing is used. This is accomplished by a nose clip, which consists of a V-shaped metal spring, the ends of which are provided with felt pads. A toothed ratchet is attached to the ends of the spring, and serves to hold the spring tightly clamped on the nostrils in the proper position (see Fig. 2).

Some individuals experience great distress when made to breathe through the mouth. For these it is best to use a face mask. Unfortunately at the present time no mask is entirely satisfactory. Perhaps the best is one sold by Siebe,

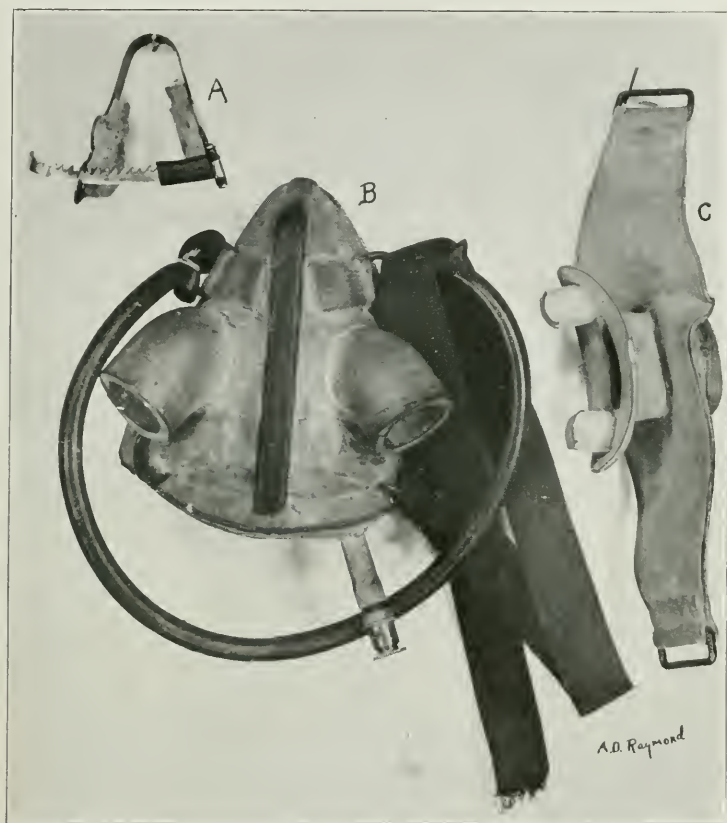


Fig. 2.—A, Nose clip; B, Face mask; C, Mouthpiece.

Gorman & Co., which is pictured in the illustration. After being placed in position the face mask should be tested for leaks, which can be done by putting soap around the edges.

*The Valves.*—The valves of Tissot are probably the best for the purpose, but they are expensive and hard to obtain. We have made perfectly satisfactory valves from the prepared casings used in the manufacture of Bologna sausage.<sup>4</sup> These can be obtained preserved in salt, and they will keep indefinitely on ice. When needed a short piece is taken, washed free from salt by allowing water from the tap to run through it, and softened in a weak glycerin solution. The

gut becomes very soft and pliable, and does not dry quickly. A piece of the casing about 10 cm. long is threaded through a glass tube of about 15 mm. bore and 4 to 6 cm. long. One end of the casing is brought around the outside of the tubing and secured by means of a thread. The lower end of the membrane is pinched off and the casing is then cut a little more than half way across its middle, so that this opening will lie just within the free end of the tube when the casing

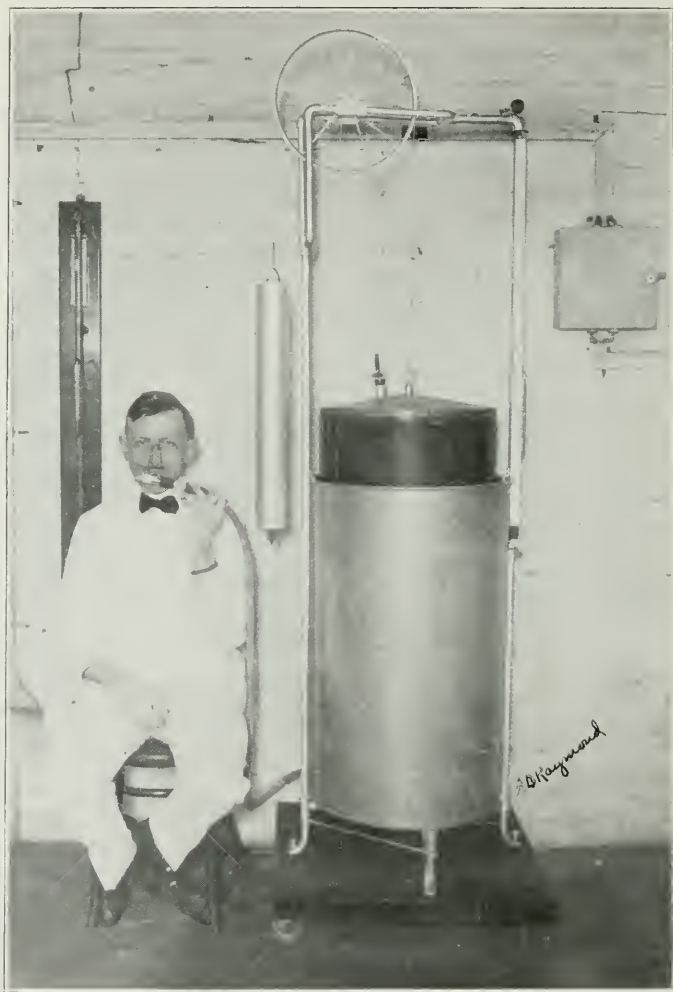


Fig. 3.—The Tissot spirometer. In actual experiment, subject is reclining or lying down and the valves and mouthpiece are held with a clamp.

is drawn back through it. The loose end of the casing is twisted slightly—an essential procedure,—and is then secured by a thread on the outer side of the tube. If properly made, the valve will work freely without vibration, and the opening will be sufficiently large to allow a good current of air to pass. It should collapse instantly and be air-tight when the current of air is reversed. The back lash, or lag of closure, of these valves is extremely small, and they will open or close with a pressure of air not exceeding the pressure changes in



normal respiration. When not in use, the valves should be kept in glycerin water on ice. Valves prepared in this way have been in use a month without loss of efficiency. They are, however, made with so great ease that new valves are provided for each subject, and they are, therefore, especially adapted to ward work.

These valves are inserted in reverse order into a supporting metal T-piece, and the joints made air-tight by tape. The stem of the T is connected with the mouthpiece. Through a rubber tube of about  $\frac{3}{4}$ -inch bore, the expired air is collected in the spirometer.

*The Tissot Spirometer.*—The Tissot spirometer is pictured in Fig. 3. We have found the 100-liter size to be very serviceable in the clinic. This instrument is mounted on a platform having rubber wheels, and can be moved about the wards with ease. The bell of the spirometer is made of aluminum and is suspended in a water-bath between the double walls of a hollow cylinder made of galvanized iron.\* The height of the bell is 72 cm., and the diameter 42 cm. An opening at the bottom of the cylinder connects through a three-way stopcock with the rubber tube leading from the expiratory valve of the mouthpiece. The bell is counterpoised by means of a weight. In the original Tissot spirometer an automatic adjustment permitted water in amount equal to the water displaced by the bell to flow from the spirometer cylinder into the counterpoise cylinder as the bell ascended out of the water. The bell, being heavier out of water than when it is immersed, is accordingly counterpoised in any position, although Carpenter<sup>2</sup> has shown that this refinement is unnecessary. An opening in the top of the spirometer permits the insertion of a rubber stopper, through which are inserted a thermometer, a water manometer, and a stopcock with tube for drawing the sample of air. A scale on the side of the instrument gives the volume of the air.

During an observation the subject sits in a reclining position or lies upon a couch. When the bell of the spirometer is placed at zero, the mouthpiece adjusted in the mouth, and the nose clamped, respiration is started, the expirations being passed through the stopcock, which is so turned as to allow them to pass to the outside air. After a few minutes the stopcock is turned so that the expirations are passed into the spirometer for a definite length of time. At the end of the period the cock is again turned, and after the barometric pressure, temperature, and volume of the air have been noted, the composition of the air is determined.

*The Douglas Bag.*<sup>2</sup>—This is made of rubber-lined cloth, and is capable of holding from 50 to 100 liters. It is fitted with straps and is easily carried on the back. In making observations the bag is placed on the shoulders and connected with the valves, the mouthpiece of which is placed in the mouth. Respirations are then commenced with the three-way valve turned so as to allow the expirations to pass directly into the outside air. After respiratory equilibrium is established, the three-way valve is turned during an inspiratory period so that the succeeding expirations may pass into the bag. The time required to fill the bag comfortably is determined with a stopwatch. The air which has

\*The instruments in use in my laboratory were made by the Cleveland Metal Products Company, Cleveland, to which we wish to extend our thanks.

been collected in the bag during the period is thoroughly mixed and passed through a meter, the temperature and barometric pressure are noted, and a sample analyzed on the Haldane gas apparatus. The bag should be emptied completely by rolling it up when nearly empty, and then allowed to fall back naturally before and after using.

*The Haldane Gas-analysis Apparatus.*—The Haldane method of analysis of expired air is simple and easily learned. The apparatus (Fig. 5) consists of a gas burette, a control burette of the same size (both surrounded with a water jacket), and bulbs containing dilute caustic potash soda solution for the absorption of the carbon dioxide and an alkaline pyrogallate solution for the ab-



Fig. 4.—The Douglas bag method for determining the respiratory exchange. The arrangement of mouthpiece, valves, and connecting tubes have been found to be more convenient than that recommended by Douglas.

sorption of the oxygen. The gas burette is connected with the bulbs by a two-way stopcock, which allows a sample of gas to pass into either bulb. The control tube is put into connection with the burette through a manometer tube, which is connected with the alkali bulb, and can be made to compensate for any changes in temperature that may occur during the course of the analysis. For an analysis the gas is transferred to the burette from the sampling tube, saturated with water vapor over mercury, and then measured, after which it is transferred into the caustic soda solution to free it from carbon dioxide, and then returned to the burette to determine the loss of volume due to carbon-dioxide absorption. It is then transferred into the alkaline pyrogallate solution,

which frees it from oxygen, after which it is again brought back to the burette to determine the loss in volume due to the absorption of the oxygen.

The detail of the Haldane apparatus is shown in Fig. 5. The measuring burette holds 21 c.c. The bulb is of 15 c.c. capacity, and the graduated stem,

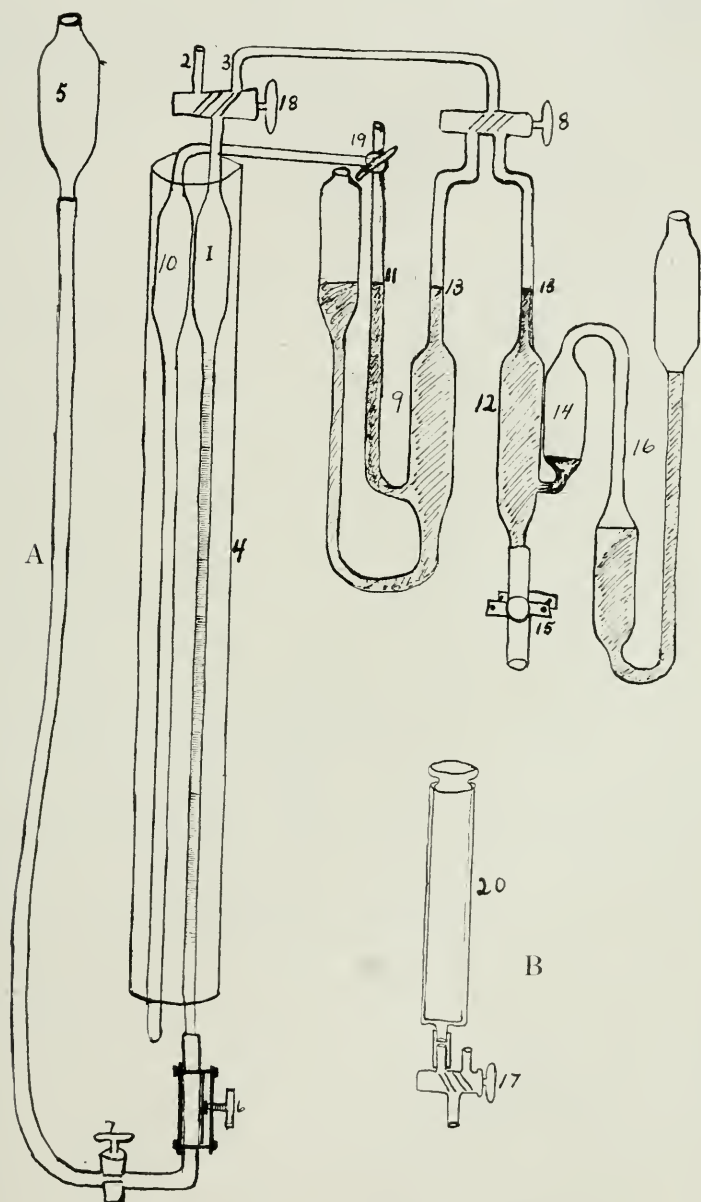


Fig. 5.—Haldane gas apparatus (A) and Pearce sampling tube (B).

which is about 4 mm. in bore and 60 cm. in length, is graduated to 0.01 c.c. from 15 c.c. to 21 c.c. The stopcock at the top of the burette is double-bored, so that in one position air can be drawn in from a gas sampler (2) and in another sent into the absorption bulbs (3). The lower part of the burette extends through

the rubber cork at the bottom of the water jacket (4). A piece of rubber tubing is attached to the bottom of the burette and placed within a metal tube, inside of which is a piece of metal which presses against the rubber tubing, the pressure being controlled by means of a fine adjusting screw (6). Below this a glass stopcock (7) connects with rubber tubing to the mercury leveling bulb (5). The absorption bulb for carbon dioxide, containing 20 per cent NaOH or KOH (9), is put in connection with the burette by suitably turning stopcocks (3 and 8).<sup>\*</sup> The control burette (10) is also in connection with this bulb through the manometer tube (11).<sup>†</sup> Any variation in temperature which may occur during the analysis will cause the level of the alkaline solution in the manometer to change.

When final readings of the shrinkage of volume are made, the level of the caustic solution is returned to the level of that in the manometer. By so doing, any error due to temperature changes is avoided, since change in temperature must be equal in the two burettes.

The absorption bulb for oxygen (12) is filled with a solution made by dissolving 10 grams of pyrogalllic acid in 100 c.c. of a nearly saturated KOH solution. The specific gravity of the KOH should be 1.55, which is obtained approximately by dissolving the sticks (pure by alcohol) in an equal weight of water. The mark (13) on the stem of the bulb indicates the level at which the solutions should stand. Enough pyrogallate solution is introduced through tube 15 to fill bulbs 12 and 14 two-thirds full, then pyrogallate solution is poured into tube 16 until the difference in level of the fluids is sufficient to produce enough pressure to raise the level of the pyrogallate solution in 12 to the level 13 on the stem. Stopcock 8 must be open during this procedure. It may be necessary to add or take away a little pyrogallate solution through 15 to attain the above level.

Care must be taken to allow for complete absorption of oxygen from the air that is entrapped between 14 and 16 before an analysis is made; otherwise changes will be produced in the level of the pyrogallate solution. The air in the capillary tubing connecting the burettes with the absorption bulbs must also be freed of carbon dioxide and oxygen. This can be accomplished by making a dummy analysis of atmospheric air before the real analysis. Great care must be taken to have atmospheric pressure in all the tubes at the start of the analysis. This is accomplished by opening the stopcock in the burette first to atmospheric air and then to the absorption bulbs, until no further change in the level of the fluids in the stems of the absorption bulbs occurs. This level is then marked and used as the standard. A small amount of water in the burette over the mercury assures saturation of the air with water vapor. Time for drainage must be allowed before making readings.

A very serviceable *sample tube* for the transfer of air can be made from a 30 c.c. ground-glass syringe, to which is attached a two-way stopcock. A cut

<sup>\*</sup>The stopcock (8) is double-bored, so that the bulb leading from the burette can be brought into connection with either 9 or 12.

<sup>†</sup>This tube also has a three-way stopcock (19), so that the tube may be opened to the outside.



of this is shown in the illustration. The dead space in these syringes is washed out by working the piston back and forth several times. A thin coating of vaseline prevents leakage of the gas. I have found that these sampling tubes will retain a sample of expired air without change up to eight hours.

*Manipulation of Apparatus.*—The sampling syringe (20) is attached to opening 2 of burette, and its stopcock (17) opened to atmospheric air as represented in the illustration. The level of the mercury is then brought to the level of the stopcock in the syringe and the stopcock is closed. The bulb of mercury is lowered so that the mercury falls in the burette. This draws the piston of the syringe with it, and fills the burette with air from the syringe. It is advisable to put a little positive pressure on the piston of the syringe in the maneuver to prevent possible leakage. When all of the air is in the burette, a slight positive pressure is produced in the burette by gently pressing on the piston, and immediately thereafter the stopcock on the syringe (17) is again turned to the original position. This allows the pressure of air in the burette to come to that of the atmosphere pressure. The height of the mercury is now adjusted to a convenient height in the burette by closing cock 7 and turning the milled screw 6. The cock 18 is now made to communicate with the absorption bulbs. If the air in the burette is at atmospheric pressure, no change will occur in the level of the fluids. The reading is then taken on the burette.

The next step in the analysis consists in turning stopcock 8 to communicate with the caustic soda solution in bulb 9, and the leveling tube 5 is raised, forcing mercury into the burette and the air into bulb 9. The gas is passed back and forth several times until absorption is complete, as can be determined by the fact that the level of the mercury in the burette remains constant when the fluid in the bulb is returned to its original level (13) on the stem. In this adjustment it is convenient to make the gross leveling by the mercury bulb and the fine leveling by closing 7 and turning 6 until the fluid in 9 is at the original height. The reading on the burette indicates the loss in volume due to the carbon dioxide absorbed.

The oxygen is removed by a similar procedure, the gas being passed into the alkaline pyrogallate solution by turning cock 8 to communicate with bulb 12. The absorption of oxygen is slower than for carbon dioxide, and more care must be taken to get complete absorption. The air in the capillary tubing between the fluid in 9 and stopcock 8 must be washed out several times in order to get the oxygen which is left in it after the absorption of the carbon dioxide. When this is complete, the final reading on the burette is made and the loss in volume from the second reading represents the oxygen.

#### THE CALCULATIONS

*The calculation of the percentile composition of the air and of the respiratory quotient* is represented in the following example of an actual analysis.

The temperature and barometric pressure as taken at the time of the experiment were 20° C. and 747 mm. Hg.

*CO<sub>2</sub> analysis—*

1st reading of burette.....	20.00
2nd reading of burette after absorption of CO <sub>2</sub> ....	19.20

CO<sub>2</sub> absorbed ..... 0.80

$0.80 \div 20 = 4.0$  per cent CO<sub>2</sub> in expired air.

Since there is 0.03% CO<sub>2</sub> in atmospheric air.

3.97% of the CO<sub>2</sub> in the expired air was derived from the blood.

*O<sub>2</sub> analysis—*

2nd reading of burette.....	19.20
3rd reading of burette after absorption of O <sub>2</sub> .....	15.90

O<sub>2</sub> absorbed ..... 3.30

$3.30 \div 20 = 16.50\%$  of O<sub>2</sub> in expired air.

*Determination of R. Q.—*

O<sub>2</sub> in atmospheric air = 20.94%

O<sub>2</sub> + CO<sub>2</sub> in expired air (16.50 + 4) = 20.50%

$100 - 20.94 = 79.06\%$  N in atmospheric air.

$100 - 20.50 = 79.50\%$  N in expired air.

Since the nitrogen is not changed in volume, the last figure shows that more oxygen must have been taken in during inspiration than O<sub>2</sub> + CO<sub>2</sub> has been given back in expiration. This obviously must be taken into account in the calculations. The amount of O<sub>2</sub> actually inspired for each 100 c.c. of air expired is found as follows:

$$\frac{20.94 \text{ (percentage of O}_2 \text{ in atmospheric air)}}{79.06 \text{ (percentage of N}_2 \text{ in atmospheric air)}} \times 79.60 \text{ (percentage of N}_2 \text{ in expired air)};$$

or  $0.265$  (constant factor)  $\times 79.5$  (percentage N<sub>2</sub> found for this observation) =  $21.07$ , the volume of O<sub>2</sub> which would have been present in expired air to account for N<sub>2</sub> present.†

$21.07 - 16.50 = 4.57\%$  O<sub>2</sub> actually absorbed.

3.97 = percentage CO<sub>2</sub> excreted.

$\frac{3.97}{4.57} = 0.87$ , the respiratory quotient, or ratio of CO<sub>2</sub> excreted to O<sub>2</sub> absorbed.

*Total Gas Exchange.*—The volume of air expired in 15 minutes into the Tissot spirometer was found to be 100 liters measured at 20° C. and 747 mm Hg. (brass-scale barometer). This volume of gas must be corrected so as to give the volume of dry air at 0° and 760 mm. Hg. To do this two things must be taken into account: (1) Since the expired air is saturated with water, the pressure due to water vapor must be subtracted from the observed barometric pressure to obtain the true pressure. The vapor tension of water for various temperatures is given in Table II. (2) The barometer tube lengthens or contracts with heat or cold, and, therefore, the barometric readings must be corrected. The corrections for ordinary barometric readings are found in

\*This is the constant O<sub>2</sub> percentage in air.

†This calculation can be simplified by the use of Table I which gives the O<sub>2</sub> figure corresponding to the various percentages of O<sub>2</sub> + CO<sub>2</sub> in the expired air.

Table III. The figure corresponding to the temperatures is subtracted from the barometric reading in order to obtain correct barometric pressure.

In the above experiment, the correction for the barometer is 2.41 mm. (see Table III), and that for vapor tension at 20° C. is 17.4 (see Table II).

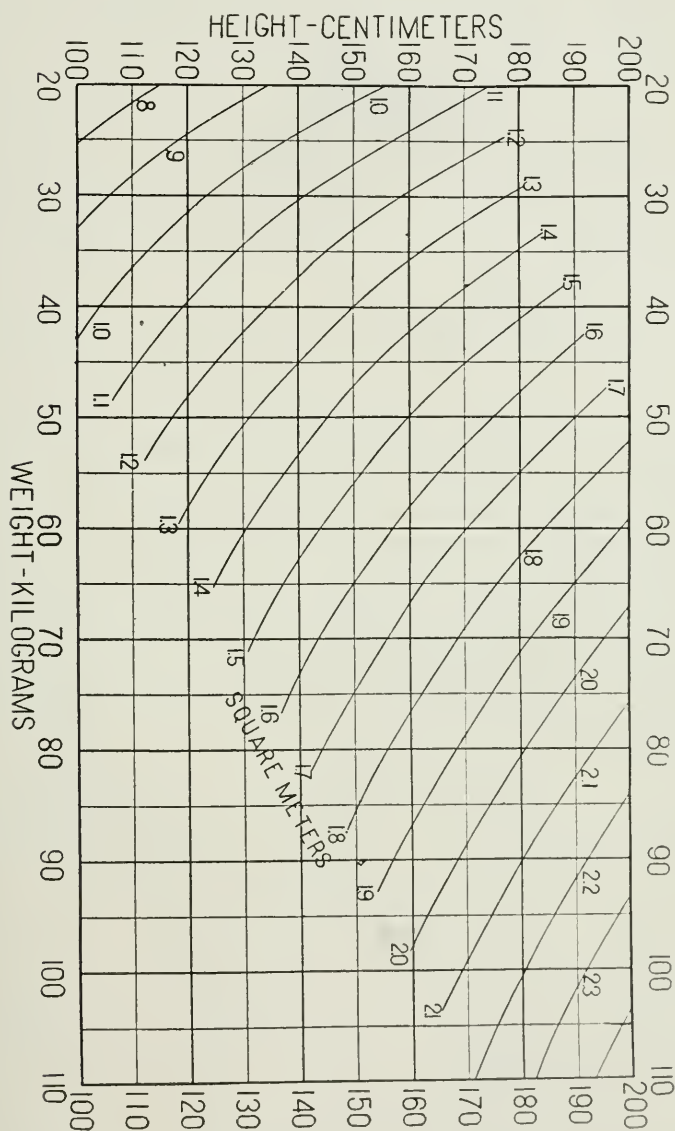


Fig. 6.—Chart for determining surface area of man in square meters from weight in kilograms (Wt.) and height in centimeters (Ht.) according to the formula: Area (Sq. Cm.) = Wt. 0.425 X Ht. 0.725 X 71.84. (From Dubois & Dubois, Arch. Int. Med., 1917, vol. 17.)

*Actual Barometric Pressure.*— $747 - (17.4 + 2.39) = 727.21$  mm. The coefficient of expansion of gases is taken as 0.003665, or  $1/273$ ; therefore, the volume of  $O_2$  equals the volume at 1° divided by  $1 + 0.003665 t$ ; and hence

$$V_o = \frac{V \times 273}{273 + t} = \frac{V}{1 + 0.003665 t}, \text{ when } V_o = \text{Volume at } 0^\circ \text{ and } V = \text{Volume at } t^\circ.$$

The volume of gas being inversely as the pressure,  $V_o = \frac{VP}{760}$ , where  $V$  = volume at  $P$  pressure; or working both corrections together,

$$V_o = \frac{VP \times 273}{760 \times (273 + t)} = \frac{VP}{760 (1 + 0.003665 t)}.$$

This formula applied to the present problem reads:

$$V_o = \frac{100 \times 727.2}{760 (1 + 0.003665 \times 20)} = 89.2 \text{ liters.}$$

The latter calculation can be considerably simplified by using standard tables which give constants for corrections of gas volumes. These are easily obtainable and are given in part in Table IV. According to these tables, for  $20^\circ$  C. and 727.21 mm. Hg. B. P., the factor is 0.89124.

$$0.89124 \times 100 = 89.124 \text{ liters, } 0^\circ \text{ C. and 760 mm. Hg.}$$

$$0.89124 \times 4.57 = 46 \text{ liters of } O_2 \text{ in 15 min., or 18.4 liters per hour.}$$

*The Caloric Value Calculated from the Gas Exchange.*—By reference to Table V, giving the heat value of 1 liter of  $O_2$  at various respiratory quotients, it is found that at a R.Q. of 0.870, 4.888 calories are expended; 18.4 liters of  $O_2$  is therefore equivalent to  $18.4 \times 4.888 = 90$  calories.

The results must be calculated for surface area as well as body weight, since the metabolism is roughly proportionate to the surface area. Suppose the subject weighed 85 kg. and was 170 cm. in height; by reference to the chart for determining the surface area of man (Fig. 6), this is found to be 1.96 square meters. The caloric expenditure per square meter in the above case is therefore  $\frac{90}{1.96} = 45.8$  calories.

TABLE I\*

THE VOLUME PERCENTAGE OF OXYGEN WHICH WOULD BE PRESENT IN THE INSPIRED AIR TO ACCOUNT FOR THE  $CO_2 + O_2$  FOUND IN THE EXPIRED AIR

% $CO_2 + \% O_2$ IN EXPIRED AIR	VOL. % OF $O_2$ IN INSPIRED AIR	% $CO_2 + \% O_2$ IN EXPIRED AIR	VOL. % OF $O_2$ IN INSPIRED AIR
19.4	21.38	20.4	21.10
19.5	21.35	20.5	21.07
19.6	21.31	20.6	21.04
19.7	21.28	20.7	21.01
19.8	21.25	20.8	20.98
19.9	21.22	20.9	20.96
20.0	21.20	21.0	20.93
20.1	21.18	21.1	20.90
20.2	21.15	21.2	20.88
20.3	21.13	21.3	20.86

\*The table gives the oxygen in the inspired air equivalent to the sum of the percentages of  $CO_2$  and  $O_2$  in the expired air.



TABLE II\*

## TENSION OF AQUEOUS VAPOR IN MILLIMETERS OF MERCURY

Temp.	15°	16°	17°	18°	19°	20°	21°	22°	23°	24°	25°
Mm.	12.7	13.5	14.4	15.4	16.3	17.4	18.5	19.7	20.9	22.2	23.5

\*To obtain the dry barometric pressure, subtract the mm. Hg. corresponding to the temperature of the air from the barometric pressure at the time of the experiment.

TABLE III\*

## TEMPERATURE CORRECTIONS TO REDUCE READINGS OF A MERCURIAL BAROMETER WITH A BRASS SCALE TO 0° C.

Temp.	700 mm.	710 mm.	720 mm.	730 mm.	740 mm.	750 mm.	760 mm.	770 mm.
15°	1.69	1.72	1.74	1.77	1.79	1.81	1.84	1.86
20°	2.26	2.22	2.32	2.36	2.39	2.42	2.45	2.48
25°	2.83	2.87	2.91	2.95	2.99	3.03	3.07	3.11

\*Subtract the appropriate quantity as found in table from the height of the barometer. The table is for a barometer with a brass scale, and the values are a little lower (about .2 mm.) than for the glass scale. The corrections for intermediate temperatures can be approximated.

TABLE IV\*

## TABLE FOR REDUCING GASEOUS VOLUMES TO NORMAL TEMPERATURE AND PRESSURE

Mm.	15°	16°	17°	18°	19°	20°	21°	22°	23°	24°	25°
720	.898	.894	.891	.888	.885	.882	.880	.877	.873	.870	.867
730	.910	.907	.904	.901	.897	.894	.891	.888	.885	.882	.879
740	.922	.919	.916	.913	.910	.907	.904	.901	.897	.894	.891
750	.935	.932	.928	.925	.922	.919	.916	.913	.910	.907	.904
760	.947	.944	.941	.938	.934	.931	.928	.925	.922	.919	.916
770	.960	.957	.953	.950	.948	.945	.940	.936	.933	.930	.927

\*The observed volume, when multiplied by the factor corresponding to the temperature and corrected pressure, will give the volume of the expired air reduced to 0° and 760 mm.

## BIBLIOGRAPHY

- <sup>1</sup>Tissot: Jour. de physiol. et de pathol. gén., 1904, vi, 688.
- <sup>2</sup>Douglas: Proc. Physiol. Soc., p. xvii, Jour. Physiol., 1911, xlii.
- <sup>3</sup>Carpenter: Carnegie Institution of Washington, Report 216, 1915.
- <sup>4</sup>Pearce: Am. Jour. Physiol., 1917, xliv, 369.
- <sup>5</sup>Haldane: Methods of Air Analysis, Chas. Griffin & Co., London, 1912.

## A HIGHLY DIFFERENTIATING POLYCHROMATIC TOLUIDIN-BLUE STAIN\*

BY MOSES BARRON, M.D., MINNEAPOLIS, MINN.

ANYONE working in a clinical-pathologic laboratory realizes the need for reliable stains that possess the virtues of precise differentiating powers, as well as simplicity and rapidity of technic. The almost countless number of stains that go to make up the armamentarium of the laboratory technician testify to the continuous efforts of many workers towards discovering serviceable stains.

For many years, I have been impressed with the polychromatic qualities of toluidin-blue. I became acquainted with the dye as an ingredient of Ponder's stain for diphtheria bacilli. I tried various combinations to increase its polychromatic properties, until one of our laboratory technicians, Richard Lundquist, suggested the idea of boiling the dye with an alkali. Heretofore, this process was used chiefly in obtaining various color compounds from methylene blue. For instance, Unna's polychrome methylene blue is prepared by boiling methylene blue with potassium carbonate. Similarly, the production of various azures is described by Tribondeau.<sup>1</sup> Goodpasture<sup>2</sup> describes a serviceable acid polychrome stain. But the polychromatic qualities obtainable from toluidin-blue seem to excel all the others in the sharpness of differentiation. Although its special usefulness lies in staining frozen sections for rapid diagnosis, it is of distinct value in a large number of routine laboratory procedures.

The preparation of the stain is as follows:

Toluidin-blue	1.0
Potassium carbonate	1.0
Distilled water	400.0

Boil solution in glass beaker until it is reduced to 300 c.c.

Cool; then add:

Toluidin-blue	2.0
Sodium chloride	3.0
Glacial acetic acid	12.0
Alcohol	15.0

Stir mixture until ingredients are completely dissolved. It is ready for use at once, and can be used over and over again. It does not precipitate and keeps indefinitely.

The following technic is employed in staining frozen sections for rapid diagnosis. A piece of tissue which is not more than 3 mm. in thickness is dropped into boiling 10 per cent formalin for about one minute. Fresh tissue without hardening may be used in many cases but the sections are more difficult to handle. The tissue is cut with the freezing microtome, and the sections

\*From the Laboratory of Pathology of the Medical School of the University of Minnesota, Minneapolis, Minn.

are placed in a dish containing physiologic salt solution. The sections are quickly straightened out and carried over into a small dish of the stain where they are left for about fifteen seconds. They can be left for a much longer time without overstaining. The sections are then transferred to another dish of physiologic salt solution where they are rapidly washed for a few seconds to remove the excess of stain, and then are at once mounted in the same solution on a slide, and a coverslip applied. The section is now ready for examination. The entire process, from the time the section is cut to the completed preparation for microscopic study, need not take more than one minute in most instances.

I have found that by handling the sections in physiologic salt solution and by having approximately 1 per cent salt in the stain, the outlines of the cells and their nuclei are clearer and sharper in the resulting preparation. The acetic acid in the stain helps to accentuate the nuclei, while the alcohol aids in straightening out the section after its transference into the last salt solution.

Sections stained by this method give approximately the following microscopic pictures. The cytoplasm of epithelial cells stains a purplish blue of varying intensity depending upon the age of the cell. The younger the cell, the deeper the stain. The stratum germinativum of the skin, therefore, takes a deeper stain than the stratum corneum. The nuclei stain deep purple. Tumor cells, like those of carcinomata, stain deep purplish blue. Mitotic figures show up very distinctly, as the chromosomes stain a purplish black. Hyaline connective tissue stains pink and the intensity of the color is proportional to the degree of hyalinization present. The nuclei of the leucocytes take a very deep purple, while the cytoplasm stains only feebly bluish. The granules of the neutrophiles stain faintly bright green. The granules of the basophiles are dark purple, almost black. Plasma cells are easily identified. The cytoplasm is a light purplish blue, while the excentric nucleus appears reticular and takes a deep purple color. An achromatic zone surrounds the nucleus. Erythrocytes do not stain but appear greenish yellow. The endothelial cells lining blood vessels and lymph spaces stain light bluish purple.

The myelin sheaths of nerve fibers are bright purple, while the cytoplasm of the cells is bluish green. Cartilage stains a deep purple. Elastic fibers are brilliant green. Cells containing fat droplets present a dirty brown appearance. Because of this the zones in the lobules of a liver involved in chronic passive congestion with fatty metamorphosis stand out very prominently. In the adrenal, the cortex appears brown while the medulla is light blue.

Atherosclerosis of the aorta gives a beautiful picture. Atheromatous patches containing fat droplets are brown; elastic fibers are brilliant bluish green; and hyalinized connective tissues are pink.

Besides the staining of tissues, the examination of stools for ova and parasites is facilitated by the use of this stain. A drop of emulsified stool is mixed with a drop of the stain on a slide, and a coverslip applied. Practically all the elements in the normal stool take a purplish red color. If ova of any of the tenia be present, they stand out as regular yellowish or golden yellow bodies against a purple background. The identification of the ova thus becomes a simple matter because they appear so different from any other elements present.

But the stain is even more valuable for identifying amebæ in stools. Hitherto a correct diagnosis of ameba could be made only through the examination of a warm stool on a warming stage. With this stain, amebæ can readily be identified by taking a drop of the mucoid portion of the stool and mixing it with a small drop of the stain. The cytoplasm of the amebæ takes on a purplish blue and the outline of the cells becomes very sharp. The nucleus often shows very distinctly. Vacuoles and ingested particles become prominent. The amebæ can be recognized with the low power by their size, regularity in form, sharpness of cell outline, and the deeper bluish tinge of the cytoplasm than that of most of the other elements present. The absolute identification can then be made by means of the high power dry lens.

Diphtheria bacilli show up excellently with this stain. The smear is flooded with the stain and steamed gently for about ten to fifteen seconds. It is then washed and dried. The granules appear a deep brilliant purplish red, while the rest of the cell bodies stain a light purplish blue.

Because of the simplicity and the quite general usefulness of this stain I feel justified in reporting its formula.

#### BIBLIOGRAPHY

- <sup>1</sup>Tribondeau, L., and Dubreuil, J.: Nouveaux colorants microscopie derives du bleu de methylene, *Compt. rend. Acad. d. sc.*, 1917, clxiv, 551-53.  
<sup>2</sup>Goodpasture, C. W.: An Acid Polychrome Methylene Blue Solution for Routine and Special Staining, *Jour. Am. Med. Assn.*, 1917, lxi, No. 12, p. 998.

## A SUBSTITUTE FOR WHITE MICE IN PNEUMOCOCCUS GROUPING

O. J. WALKER, M.D., AND W. J. BRUCE, OKLAHOMA CITY, OKLA.

PNEUMONIA is at all times a very prevalent disease during the winter months, and, if anything, it has been more widespread than ever this year. Especially in our army camps it has appeared in almost epidemic form.

Every effort has been made to combat this dread disease, and of no small avail has been the new treatment with the antipneumococcic serum produced by the workers at Rockefeller Institute. As this method has been published in detail in many of the current journals, it needs no repetition here.

This serum treatment depends upon the exact diagnosis of the specific type of the pneumococcus in each individual case. The method as devised by Cole<sup>1</sup> and his coworkers calls for a pure culture of the infecting pneumococci in the shortest possible period after the case is seen. This pure culture is necessary for the performance of the agglutination and precipitin tests with the known immune serums of Types I, II, III, and the consequent determination of the type of organism causing the infection. Owing to the high susceptibility of white mice to the pneumococcus, this purpose was found to be best and most quickly attained by the intraperitoneal injection of a small amount of sputum into one of these animals. A pure culture can be obtained in this way in from six to sixteen hours.



Because of the unusually large numbers of cases of pneumonia this winter, and because of the comparative newness of the method, breeders of white mice have not only raised the price of their animals to a ridiculous figure, but have been unable to adequately supply the demand.

To meet this emergency O. T. Avery<sup>2</sup> devised a quick method for grouping the pneumococcus which was independent of the use of white mice. However, he recommends the method only as an emergency, and states that it is not as reliable as the mouse method.

Being unable to procure the white mice in sufficient quantities for our work, we decided to try the common brown house mouse. For purposes of comparison of their relative susceptibility, white and brown mice were injected in duplicate with similar amounts of the same suspension of sputum. In every case the tissues of the wild mouse proved to be more susceptible to invasion by the pneumococcus as measured by the time of death and the relative number of pneumococci in the peritoneal exudate at a given time after the injection. In every case the agglutination and precipitin reactions were just as clear cut with the peritoneal washings of the brown mouse as with the white mouse.

The ordinary brown wild mouse can be obtained anywhere and at any time for the cost of trapping. We used for this purpose the small wire cage trap that can be purchased for twenty-five to thirty-five cents at almost any hardware store. A half dozen of these traps distributed among a few friends will quickly supply sufficient animals for a day's work and the supply is almost inexhaustible.

The wild mouse is more difficult to handle than his white brother, as he fights and bites as the little fiend he is. However this difficulty is easily overcome by wearing a pair of heavy leather gloves.

We report our experiences in this work in the hope that it may help others as they have certainly helped us in the present dearth of white mice.

#### BIBLIOGRAPHY

<sup>1</sup>Monographs of the Rockefeller Institute for Medical Research, No. 7.

<sup>2</sup>Avery, O. T.: Determination of Types of Pneumococcus in Lobar Pneumonia: A Rapid Cultural Method, Jour. Am. Med. Assn., lxx, No. 1, p. 17, 1917.

# *The Journal of Laboratory and Clinical Medicine*

Vol. III.

APRIL, 1918

No. 7

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.  
Ann Arbor, Mich.

## ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D.	- -	ST. LOUIS
HANS ZINSSER, M.D.	- - -	NEW YORK
PAUL G. WOOLLEY, M.D.	- -	CINCINNATI
FREDERICK P. GAY, M.D.	- -	BERKELEY, CAL.
J. J. R. MACLEOD, M.B.	- - -	CLEVELAND
ROY G. PEARCE, M.D.	- - -	CLEVELAND
ROGER S. MORRIS, M.D.	- - -	CINCINNATI
GERALD B. WEBB, M.D.	-	COLORADO SPRINGS
E. E. SOUTHARD, M.D.	- - -	BOSTON

Contents of this Journal Copyright, 1918, by The C. V. Mosby Company—All Rights Reserved  
Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

## EDITORIALS

### *Functional Heart Tests*

THE application of polysphygmographic, and more particularly of cardiographic, technic in the clinical diagnosis of cardiac disease has served to reveal the exact nature of many conditions which previously, in our ignorance, were classified loosely as functional cardiac disorders. No other physical sign of disturbed heart action could be detected than an alteration in rate or an irregularity in rhythm: no bruit or hypertrophy could be made out, although subjective symptoms of a decidedly alarming character might be prominent. The discovery of heart block, of auricular fibrillation and flutter, and more recently of the various so-called bundle lesions, has opened out an entirely new field in cardiac medicine and therapeutics. But even when every refinement of diagnosis is applied, there yet remain many cases in which there is perfectly evident cardiac embarrassment with no demonstrable physical sign of unphysiologic heart action. Irritable heart and so-called soldier's heart are examples of such cases.

The problem which most pressingly calls for further investigation at present

is, therefore, the development of methods by which the functional capacity of the heart can be gauged. Experimental investigation by Starling and his pupils with heart-lung preparations, in which the work by the heart may be accurately measured, had shown that the dominating factor is the venous inflow. If this remains constant, neither a great acceleration in heart rate nor an increase in arterial blood pressure can in itself alter the outflow. But the extent to which alterations in venous inflow may correspondingly alter the arterial discharge is so considerable as to permit the conclusion to be drawn that the reserve power of the heart muscle is equal to, if not greater, than the power which the heart actually expends on the performance of its ordinary pumping action. It is this reserve power which constitutes the factor of safety of the heart, and which may become narrowed down without any evident signs of cardiac weakness until some stress is thrown on the organ.

To test the extent of this factor of safety of the heart is the object of some recent investigations in the cardiac clinics. The adoption of suitable methods for the test involves two questions: first, how we are to gauge the heart's reaction, and secondly, how we are to subject the organ to approximately measurable increasing amounts of extra strain. Experience has shown that the first object is satisfactorily fulfilled by measuring the heart rate (pulse) and the systolic blood pressure, and that the second is best applied by graded muscular exercise.

Elsewhere in this JOURNAL will be found an explanation of the probable nature of the mechanism by which the cardiovascular system responds to increasing effort on the part of the somatic musculature, but at present we need concern ourselves merely with the readily measurable changes by which the response can be gauged. Rapport,<sup>1</sup> Barringer,<sup>2</sup> and Cotton, Rapport and Lewis,<sup>3</sup> have shown that the pulse rate and systolic blood pressure taken at frequent intervals immediately after the exercise is discontinued, furnish the most practical guides. The pulse rate may be taken either by the ordinary, palpatory, method (Barringer) or by applying to the opposite arm to that from which blood pressure is being measured a blood-pressure armlet connected with an Erlanger capsule and a polygraph; the tubing leading to the polygraph is disconnected from the capsule during the exercise, but is immediately attached when this is ended. The blood pressure is taken by auscultatory or palpatory methods, the cuff being left in position on the arm during the exercise.

It has been found that the determining factor in the heart's response does not depend on the particular group of contracting muscles, but on the actual amount of work performed (i. e., the foot pounds of work). Lifting dumb bells, walking a certain distance, or climbing stairs at a certain rate are all equally suitable methods of investigation. The most practical method would appear to be to "lift twenty-pound dumb-bells from the floor to the full stretch of the arms above the head, swinging them in one motion up and in one motion down, the complete movement occupying two, three, four or more seconds (guided by a metronome)." The movement is repeated from seven to sixty times, and its rate is governed according to the capacity of the subject, the limit being taken as the exercise that is enough to induce distress of breathing

and some fatigue. Each individual repeats the exercise on different days, and curves averaging the results obtained at each test are taken as characteristic for that individual.

Both pulse rate and blood pressure are of course increased during the exercise, and it is with regard to the immediate after-effect that the tests are concerned. Barringer states that in normal persons both pulse and pressure rapidly return to normal when the first observations are taken within 30 seconds after the work, unless an amount of work has been performed that is in excess of the heart's capacity, in which case the blood pressure does not reach its maximal height until later than 30 seconds after the work is discontinued, although meanwhile the pulse rate has returned almost to normal. According to this author the delayed rise in blood pressure always occurs after a certain amount of work (measured in foot pounds), and it does not vary in a given individual from day to day and is independent of the muscle group performing the work. But, whereas, in normal persons several thousand foot pounds of work can be done without the delayed rise, a few hundred foot pounds will cause it to appear in a cardiac case. Improvement in the heart's action is evidenced by finding that the amount of work which can be performed before a delayed rise ensues becomes greater and greater. In brief, then, Barringer believes that the "delayed rise in systolic pressure indicates that the preceding work has exceeded the limit of the heart's reserve power."

The results of Rapport, Cotton and Lewis do not entirely agree with those of Barringer. By taking the pressure measurements at more frequent intervals (the first one being taken from 3 to 10 seconds after the exercise), these authors have found that the blood pressure is usually only a little above the normal immediately after the exercise, but that it then invariably mounts to a certain level which is more or less proportional to the amount of the preceding exercise, and which is reached in from 2 to 60 seconds after the discontinuance of the work. The pressure thereafter gradually declines, to reach the normal in from 1 to 4½ minutes. In general the curves of blood pressure are identical in form for normal and cardiac cases. Such differences as exist are quantitative and not qualitative. Thus, untrained, normal subjects, by lifting twenty-pound bells forty to sixty times in double the number of seconds will usually cause the pressure to rise to about 160 mm. Hg. and the pulse to about 170 per minute. A cardiac case will show equal responses after much less work, or, if the work be the same, both pulse and pressure will rise much higher and take a much longer time to return to normal. These investigators, in contradiction to Barringer, find, however, that the summit of the blood pressure curve is not delayed.

It does not greatly concern us at present as to which of the investigators has most accurately described the essential characteristics of the responses. The important thing is that another method for testing the efficiency of the cardiovascular mechanism should be available, and one which requires no such high degree of technical skill as the polygraph and electrocardiograph. Even counting of the pulse alone following exercise supplies important information. This was shown some years ago by Pembrey and Todd.<sup>4, 5</sup> These authors found that



there is a marked difference in the time required, after a short bout of strenuous exercise (running up and down stairs for thirty seconds), for the pulse to return to its normal rate in athletically trained as compared with untrained men. Immediately after the exercise in the former group the rate was about 120, and it had become normal again five minutes later. In untrained men, on the other hand, the rate increased to about 160, and was still about 96 five minutes later. Meakins and Sanson<sup>6</sup> have found this method useful in the clinic. Any type of simple exercise can be adopted, but it must always be of the same degree so that comparison of the reactions of different individuals may be possible. Marching a certain distance at quick time followed by stair climbing (eighteen feet) furnished the exercise. Before the exercise a sphygmograph was attached to the wrist, with the patient sitting, and a tracing taken from which the pulse rate was counted during six-second periods. The sphygmograph was disconnected but the wristlet left in position during the exercise, and immediately after it the patient was again made to sit and the sphygmograph reapplied. The important point in the technic is that the pulse-counting can be done accurately for short periods of time (six seconds) immediately following the exercise. The counting was continued until the rate per minute, as estimated from six-second periods on the tracing, had returned to normal. It was found in cases of irritable heart, not only that the pulse rate was greater both immediately before and after the exercise, but—and most significantly—it took much longer than usual for the rate to regain the normal after discontinuance of the exercise. The palpitation experienced by the cardiac cases was in direct proportion to the length of time the pulse rate took to return to normal. In general the cases in which the pulse rate returned to normal within one minute had little or no cardiac distress. The importance of the method rests in its simplicity, as the patient can be observed readily from day to day and the effect of graduated exercise on the heart's efficiency properly studied.

The investigations are such as to indicate that simple quantitative tests applied following exercise are of decided value in testing the functional capacity of the heart. It would of course be desirable that all investigators should adopt uniform standards, not only for the exact type of exercise employed, but also for the methods used and the frequency of measurement.

## BIBLIOGRAPHY

- <sup>1</sup>Rapport, D. L.: The Systolic Blood Pressure Following Exercise, with Remarks on Cardiac Capacity, *Arch. Int. Med.*, 1917, xix, 981.
- <sup>2</sup>Barringer, T. B.: Studies in the Heart's Functional Capacity, *Arch. Int. Med.*, 1917, xx, 829.
- <sup>3</sup>Cotton, T. F., D. L. Rapport and Thomas Lewis: After Effects of Exercise on Pulse Rate and Systolic Blood Pressure in Cases of Irritable Heart, *Heart*, 1917, vi, 269.
- <sup>4</sup>Pembrey, M. S.: Further Advances in Physiology (E. A. Arnold), 1909, p. 228.
- <sup>5</sup>Pembrey and Todd: *Proc. Physiol. Soc., Journ. Physiol.*, 1908, xxxvii.
- <sup>6</sup>Meakins, J. C., and Sanson, G. B.: The Pulse Rate After a Simple Test Exercise in Cases of Irritable Heart, *Heart*, 1917, vi, 285.

—J. J. R. M.

*Colonel George E. Bushnell, Medical Corps*

MEMBERS of the medical profession of this country are all familiar with the names of brethren famous in medical schools in large cities and with those prominent in medical literature. Few physicians knew that the best and most learned authority on pulmonary tuberculosis was to be found in the Medical Corps of the Army.

About a year ago when the United States entered the great war for liberty, reports were reaching us that pulmonary tuberculosis was widespread among the French troops and alarming among the civilian population of France. Would our army face similar danger was at once the thought of those interested in this disease.

The Surgeon-General was fortunate in having at hand an adviser almost unknown to the general medical profession but known to them today as a master in this disease, Colonel George E. Bushnell. For fourteen years at the army tuberculosis institution at Fort Bayard, New Mexico, Bushnell had been studying and investigating in a most scholarly manner the field of tuberculosis. There was certainly no want of preparedness in this field when war came.

In June, 1917, a paper by Bushnell,<sup>1</sup> entitled "Tuberculosis in the Military Service," appeared in *The Military Surgeon*. This paper was recently described by Sewall<sup>2</sup> as "replete with learning and analytical acumen." Revealing profound thought and study, it has furnished the knowledge and counsel by which upwards of a million men have been examined by members of the Medical Reserve Corps constituting Tuberculosis Boards.

Bushnell, a graduate in arts and in medicine of Yale University, has been in the Medical Corps since 1881. In 1901, while on duty in the Surgeon-General's office, he developed pulmonary tuberculosis and spent a short time at Asheville, N. C. In 1902 he was placed on duty as surgeon at Fort Logan, Colorado, and the following year assumed command at Fort Bayard. From his colleague, Colonel Bruns, we learn that while at Fort Bayard, Bushnell introduced the open air and rest treatment for tuberculosis, practically rebuilt the entire hospital, planned all the landscape gardening, raised a great many of the trees from seed, and worked incessantly, developing every phase of the institution.

A great service was rendered to the country generally when a few years ago Bushnell<sup>3</sup> published an important contribution to counter the growing public fear of tubercle infection in adult life. A recent publication<sup>4</sup> by the same writer shows how exaggerated too were the first reports of tuberculosis that reached us from France. The clinical investigations of the healthy, as well as the diseased, led Bushnell<sup>5, 6</sup> to the correct understanding and interpretation of marginal rales, sternal rales, etc. These were signs that had long confused many physicians here and abroad as can well be recognized by consulting the literature.

The country has been most fortunate in having Bushnell called to head the Department of Internal Medicine in the Surgeon-Generals' office at the outbreak of the war, and the medical men who have been connected with this department have met with most considerate and kindly treatment and have

recognized the guiding hand of a master. Though he retired from active service on account of age, Sept. 10, 1917, it is a great satisfaction to know that Colonel Bushnell is still in charge of this most important work.

It must be a wonderful gratification to this medical officer that after a life of preparation for such a role the country's call came for the exercise and help of his many natural and acquired talents.

*"Non sibi sed toti."*

#### BIBLIOGRAPHY

- Bushnell: Tuberculosis and the Military Service, Mil. Surgeon, June, 1917.  
 Sewall: What Constitutes a Diagnosis of Tuberculosis Sufficient for Rejection for the Army?, Colorado Medicine, Nov., 1917.  
 Bushnell: Immunity Through Tuberculosis Infection, Mil. Surgeon, 1913, xxxii.  
 Bushnell: Lessons from the War as to Tuberculosis: The Situation in France, Jour. Am. Med. Assn., March 9, 1918.  
 Marginal Rales. Some Extrapulmonary Sounds which Simulate Rales, Med. Rec., New York, Jan. 20, 1912.  
 Marginal Sounds in the Diagnosis of Pulmonary Tuberculosis, Med. Rec., New York, Dec. 21, 1912.

—G. B. H.

### *Further Researches on the Physiology of the Adrenals*

A YEAR ago an editorial in these pages reviewed the work of Stewart and Rogoff on the physiology of the adrenals. Using the denervated iris, which responds with dilation to very small doses of adrenalin as a test for epinephrin in the blood, these authors were unable to confirm the general impression which recent work had created, that the adrenals, besides continually secreting some epinephrin into the blood, act as auxiliary organs to the lymphathetic system and in times of stress pour out an increased amount of epinephrin into the blood, producing thereby an increase in the blood pressure and muscular power, and decreasing the clotting time of the blood. They have since then published a number of researches which add much to our knowledge of the adrenals, and also throw additional doubt on the validity of the current theories of adrenal function.

Stewart and Rogoff have sought for the various factors which are alleged to influence the secretion of epinephrin by collecting the blood flowing from the adrenal glands in a pocket made from the lower portion of the vena cava and testing for epinephrin by the classic methods, viz: the inhibition of the movements of the rabbit's intestine and the contraction of the rabbit's uterus. They find that the rate of secretion of epinephrin is, under ordinary conditions, very constant, and is not influenced by painful stimulation of large nerves or by asphyxia. The concentration in which it occurs in the blood is, however, found to be inversely proportional to the amount of blood flowing through the adrenals, and for this reason they point out that any calculation of the amount of epinephrin secreted must not be based on the concentration found in a sample of blood issuing from the adrenal vein without taking into consideration the amount of blood flowing through the adrenals.

Cats in which the nerve supply to the adrenal glands had been severed remained well and apparently normal for as long as five weeks, when they were purposely sacrificed. The epinephrin reactions obtained on the iris or on the blood pressure showed an absence of the secretion. The authors believe, therefore, that epinephrin is not indispensable to the body, and moreover that its secretion is entirely under nervous control.

They have also investigated the alleged relationship of the secretion of epinephrin to certain hyperglycemias, and in cats in which the secretion of epinephrin was rendered impossible through section of the splanchnic on one side and the removal of the adrenal on the other, they have failed to find evidence that hyperglycemia produced experimentally by anesthesia, asphyxia, or by alleged emotional stress, is less than in normal animals.

The normal physiologic function of epinephrin, if it has any, is apparently still in the dark. The simple and highly active hormone which was thought to regulate and augment so many and such varied physiological processes, becomes again a physiologic problem.

For further details see paper by Rogoff in the January issue of this JOURNAL.

—R. G. P.

---

### *Acapnia and Shock*

**S**HOCK is a condition of low vitality bordering on death. Of its many symptoms some are primary, in the sense that they are dependent upon the initial disturbance causing the condition, and some are secondary, being merely the inevitable consequence of the physiologic depression. The first step in the investigation of the cause of shock hinges on a correct differentiation between the two classes of symptoms. Low blood pressure, nerve cell fatigue, acidosis depletion of the alkaline reserve, etc., to name only a few of the prominent symptoms, has each in turn been exploited as the primary cause of shock, but although all of these symptoms may be causative in a certain sense, no one can be pointed to as occupying so conspicuous a position in this regard that remedial means, capable of removing it thereby also cause recovery from the shock itself. The blood pressure, always low, may be temporarily raised and the patient so long as the restoration lasts, or the administration of alkali, as bicarbonate may, as Cannon reports, produce definite improvement; but even so, this does not indicate that the cause of shock is the low blood pressure or the bicarbonate deficiency. When we maintain life for sometime longer in an animal moribund from starvation by placing it in an incubator so as to prevent fall in body temperature, we do not thereby prove that the fall of temperature is the cause of death. In a complicated clinical condition like shock, where there are many conspicuous subjective symptoms, each new objective symptom, as it is discovered by the application of more refined means of diagnosis, is certain to be heralded as the progenitor of all the other symptoms; it is pronounced the cause of shock.

And there is another aspect of the problem which one must not pass by,



namely, the failure to explain why it is that the particular symptom that is supposed to be "the cause" should not infrequently be prominent in other diseases in which, however, "shock" is absent. An animal with the spinal cord severed above the level at which the vasoconstrictor fibers leave, and in which therefore the blood pressure is extremely low, shows no symptom of shock in the portions of the body anterior to the lesion. Neither is there shock when the alkaline reserve of the blood is greatly depleted, as in severe nephritis or diabetes. Other symptoms, it is true, develop but they are not those of shock.

These preliminary remarks will serve to introduce the main subject of this editorial—the relationship of acapnia to shock. Some ten years ago Yandell Henderson announced that shock is closely dependent upon a deficiency in the amount of carbonic acid in the blood, usually brought about by excessive removal of  $\text{CO}_2$  by way of the lungs; a "blowing off" of the volatile acid of the blood. It was claimed that, in the shock following stimulation of sensory nerves, the "blowing off" occurs because of the hyperpnea thereby induced, whilst in abdominal operations diffusion of  $\text{CO}_2$  from the exposed peritoneum into the air is an important factor. The proof offered in support of these claims was, partly, that the blood analyzed by the Haldane-Barcroft apparatus contained a low per cent of  $\text{CO}_2$ , and partly, that the shock could be prevented during excessive pulmonary ventilation by maintaining a fair percentage of  $\text{CO}_2$  in the inspired air, or in abdominal operations by taking precautions to prevent diffusion of  $\text{CO}_2$  from the exposed viscera. The investigations showed that a low content of  $\text{CO}_2$  in the blood is at least one of the prominent accompaniments of certain forms of experimental shock, but that it is not the cause of the condition as met with in man, was amply shown by other observers, on the one hand, by the fact that shock may develop in cases where the breathing instead of being exaggerated is from the beginning infrequent and shallow; and on the other, by the failure to remedy the shock by causing the patient to respire in a  $\text{CO}_2$ -rich atmosphere. Temporary improvement accompanied by increased respiration might result from this treatment, an observation which is no more remarkable than that the respirations in many cases of Cheyne-Stokes (periodic) breathing should become regular when the patient is made to respire in a  $\text{CO}_2$ -rich atmosphere. In such a case it is recognized that we do no more than temporarily excite the depressed respiratory center; we do not remove the cause of the depression; we only alleviate one symptom resulting from it.

For these and many other reasons the acapnia theory of shock was dignified some eight years ago by an apparently adequate burial, but from the remains has now sprung into being a new, reincarnated acapnia theory, with its birthplace in the pages of one of our most excellent scientific journals, the *Journal of Biological Chemistry*.<sup>1</sup> Surely the reincarnated infant is blessed by the environment of its birth.

In its revised form, acapnia is defined as a deficiency not merely in the amount of  $\text{CO}_2$  in the blood, but also in the capacity of the blood to absorb this gas. Under the old definition it was evidently assumed that there might be a deficiency of  $\text{CO}_2$  without an accompanying curtailment of  $\text{CO}_2$ -binding power of the blood, and acknowledgment is made to Van Slyke and his collaborators

for "the conception" of the alkaline reserve which has prompted the new definition. In so far as acapnia acts as a causative factor in shock, it would appear from the new definition that it is the depletion of alkaline reserve rather than the diminution in actual  $\text{CO}_2$  content that is important. It is, however, somewhat difficult to see from their papers what the authors really mean and their results do not contribute any facts which were not perfectly self-evident in the previous papers of Henderson himself.

Briefly stated the experimental procedure adopted in the present research consisted in removing arterial blood from dogs subjected to various conditions that we know to cause increased or diminished excretion of  $\text{CO}_2$  by way of the lungs, and determining in this blood first, the actual  $\text{CO}_2$  content, and secondly, the  $\text{CO}_2$  content after exposing the blood in a suitable apparatus to an atmosphere containing a known percentage of  $\text{CO}_2$ . Comparison of the results of the two determinations indicates to what extent the  $\text{CO}_2$  absorbing power of the blood is satisfied, whereas, those of the second method taken alone give us the same information as the now well-known Van Slyke method—namely, the reserve alkalinity or, as the authors call it, the  $\text{CO}_2$  capacity of the blood. The alveolar air was also collected for analysis in a few of the experiments by the uncertain Higgins-Plesch method. In the first series of experiments, the effect of raising the  $\text{CO}_2$  of the blood was investigated when the  $\text{CO}_2$  tension of the alveolar air was raised either by decreasing the extent of pulmonary ventilation through depression of the respiratory center by means of morphine, or by causing the animals to respire into a bag containing 5 or 6 per cent  $\text{CO}_2$ . The increase in the  $\text{CO}_2$  of the alveolar air promptly caused an increase in the  $\text{CO}_2$  content of the blood, followed later by an increase in  $\text{CO}_2$  capacity. In only one of the experiments, and in this case the rectal temperature was very low, did the capacity come up to the actual amount even after several hours. In other words, there was constantly present in the blood a ratio between  $\text{H}_2\text{CO}_3$  and  $\text{NaHCO}_3$  which was distinctly greater than  $\frac{1}{20}$ , under which conditions, as R. W. Scott<sup>2</sup> has shown in this laboratory there is a readily recognizable increase in the H-ion concentration of the arterial blood. In one experiment (No. 10) the respired air contained a very high percentage (20) of  $\text{CO}_2$ , so that the total  $\text{CO}_2$  of the blood rose to 80 and the  $\text{CO}_2$  capacity to 74; and when the breathing bag was removed and air again respired, the animal underwent apnea, because, it is said, "the alkali content of the blood had been raised so high that, when the abnormally high pressure of  $\text{CO}_2$  was withdrawn, the respiratory center lacked an adequate stimulus."

These experiments bring out absolutely nothing new. Zuntz many years ago showed that, when  $\text{CO}_2$  is added to blood the bicarbonate content is increased, and more recently R. W. Scott<sup>2</sup> by comparison of the H-ion concentration and the  $\text{CO}_2$  content demonstrated that, when the total  $\text{CO}_2$  content of the blood is raised by breathing  $\text{CO}_2$ -rich air, the ratio between  $\text{H}_2\text{CO}_3$  and  $\text{NaHCO}_3$  becomes raised but the increase in  $\text{NaHCO}_3$  lags behind that of  $\text{H}_2\text{CO}_3$ . Under the conditions of Henderson's and Haggard's experiments, therefore, there must have been an elevation in the H-ion concentration of the blood—a carbon-dioxide acidosis.

In the next paper experiments are described in which the  $\text{CO}_2$  content of the blood was experimentally lowered, and it is shown that the  $\text{CO}_2$  capacity also became depressed. To lower the  $\text{CO}_2$ , hyperventilation was induced by administering ether in such a way that there was constant hyperpnea, which "washed out" or "blew off" the  $\text{CO}_2$  of the blood. If this was allowed to go on until the  $\text{CO}_2$  content and capacity had fallen to about 33, irrecoverable shock occurred. When the ether was properly given, so as to produce deep anesthesia without preliminary hyperpnea, no decrease in the  $\text{CO}_2$  content or capacity, rather an increase, was observed, but no shock. Nor was there any shock produced by faulty etherization when the respired air contained  $\text{CO}_2$  in sufficient percentage to maintain the normal level of  $\text{CO}_2$  content and capacity in the blood. Even when a considerable depression had already occurred, the normal level could be restored by breathing a  $\text{CO}_2$ -rich atmosphere, provided the depression had not reached to from 33 to 36 per cent  $\text{CO}_2$ .

These experiments again are merely confirmatory of those of Milroy,<sup>3</sup> who showed that a definite fall in hydrogen ion of the blood resulted from forced ventilation, which, however, was not the case when the air was rich in  $\text{CO}_2$ . It necessarily follows, as has been explained above, that there must be a fall in  $\text{NaHCO}_3$  under these conditions. The point which the authors would particularly emphasize is that ether *per se* does not produce "acidosis" by some obscure effect on metabolism. Why this fact should be given more prominence than that a condition of alkalosis is established, is difficult to comprehend. Is it meant by the authors that the process by which the alkali leaves the blood is dangerous or is it the actual decrease of the absolute amount of alkali in the blood? It is much more likely that the relative excess of  $\text{NaHCO}_3$  over  $\text{H}_2\text{CO}_3$ , causing depression in  $\text{C}_H$ , is in part responsible for the shock-like symptoms.

Papers are also contributed to show the already well-known fact that excessive artificial respiration induced in other ways lowers the  $\text{CO}_2$  content of the blood and of course the  $\text{CO}_2$  capacity, there being at the same time a fall in arterial pressure with the reflexes at first hyperactive and later suppressed. When the hyperventilation is conducted with air containing 6-8 per cent  $\text{CO}_2$ , on the other hand, the blood  $\text{CO}_2$  does not fall so long as this air is breathed. Only one experiment of this last-mentioned type is reported, however, and in this it is shown that, although the blood pressure, as would be expected, rises during the forced ventilation, it falls immediately thereafter. The authors attribute the fall to chloretone, whereas the fall in the other experiment, also on chloretonized animals, is attributed to shock. Why the difference?

It is natural to conclude that since excessive hyperventilation of the lungs by artificial respiration can in itself depress the blood pressure, so also will the hyperpnea produced by overactivity of the respiratory center as a consequence of stimulation of sensory nerves. The sensory nerves stimulated were those of the peritoneum around the duodenum in animals anesthetized by morphine and believed therefore not to experience any pain. The usual results were of course obtained. If the air breathed contained a high percentage of  $\text{CO}_2$ , however, the blood pressure remained normal for a longer time (in two experiments), although in one of the experiments (No. 1) a fatal condition of shock, with con-

gested intestines, etc., supervened (there was, however, some hemorrhage in this animal).

In short, the whole series of papers is a repetition of work already published by Henderson himself, the only addition being that the alkaline reserve or, as the authors prefer to term it, the total  $\text{CO}_2$  capacity of the blood is examined with results that are inevitable from the accurately controlled work of other observers. That depression in the  $\text{CO}_2$  content and therefore the  $\text{CO}_2$  capacity of blood occurs in certain experimental conditions simulating shock as met with in the clinic, and that it does not occur when this depression is artificially prevented is exactly the same evidence as was advanced some years ago in favor of the theory that the acapnia is a causative factor in shock. There is no reply to the many objections that have been raised to the hypothesis, for example, why it should be that the blood of patients in shock often contains a practically normal  $\text{CO}_2$ -content (Rendel Short), why shock may occur after forced artificial respiration in animals even when the  $\text{CO}_2$  content of the blood is kept high and conversely, why it should often be absent when the  $\text{CO}_2$  of the blood is persistently very low, why it often occurs early in patients ( $\frac{1}{2}$  hour) in whom very little hyperpnea is present, and in patients anesthetized by a face mask (Clover's) which would cause them to rebreathe considerable percentages of  $\text{CO}_2$ , and so on.

From the biochemical standpoint, the conclusion may, however, be drawn that excessive depletion of free  $\text{CO}_2$  of the blood will cause a temporary condition of alkalosis, because it will take some time before the alkaline reserves will lower themselves to the new level. Whether this in itself can bring on a condition of shock is doubtful, for we have repeatedly injected enormous quantities of alkali, sufficient to lower the H-ion concentration for considerable periods of time, without any shocklike symptoms supervening. During this period of lowered H-ion concentration it is natural to expect that there should be a depression of many physiologic functions and particularly of the nerve centers whose tone or activity is known to be highly sensitive to H-ion concentration. The condition, however, is not shock. The temporary alkalosis can be remedied by adding acid to the blood, and probably the most convenient way to do this is by inhalation of  $\text{CO}_2$ -rich air, although exactly the same results are attained by injecting faintly acidified saline solution intravenously.

We would sum up by saying that this new work of Henderson and Haggard is a repetition of experiments of the same nature as those by which it was attempted to show that acapnia is an important causative factor in shock. No new fact is contributed to controvert the many objections that have been raised against the theory.

#### BIBLIOGRAPHY

- <sup>1</sup>Henderson, Yandell, and Haggard, W. H.: Respiratory Regulation of the  $\text{CO}_2$  Capacity of the Blood, *Jour. Biol. Chem.*, 1918, xxxiii, 333, 345, 355, and 365.
- <sup>2</sup>Scott, R. W.: The Effect of the Accumulation of Carbon Dioxide on the Tidal Air and the H-ion Concentration of the Arterial Blood in the Decerebrate Cat, *Am. Jour. Physiol.*, 1917, xliv, 196.
- <sup>3</sup>Milroy, T. H.: Changes in the Hydrogen-ion Concentration of the Blood Produced by Pulmonary Ventilation, *Quart. Jour. Exper. Physiol.*, 1915, viii, 141.

—J. J. R. M.



# *The Journal of Laboratory and Clinical Medicine*

VOL. III.

ST. LOUIS, MAY, 1918

No. 8

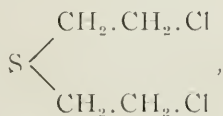
## ORIGINAL ARTICLES

### THE PATHOLOGY OF THE SKIN LESIONS PRODUCED BY MUSTARD GAS (DICHLORETHYLSULPHIDE)\*

BY ALDRED SCOTT WARTHIN, PH.D., M.D., AND CARL VERNON WELLER, M.D.,  
ANN ARBOR, MICH.

THE great World War has been epoch-making in many ways and in no way more remarkable than in the introduction of poisonous gases as agents of warfare. First used with notable effects by the Germans in the Spring of 1915, they have since been employed by the Allied forces as well. Gas attacks have become frequent features of the conflict on both sides; and as a military agent poison gas may be regarded as more effective than the use of artillery. "Gassing" has come to be more feared than the missiles of warfare. Many kinds of poisonous gas have been used by the Germans, some of them, no doubt, more or less experimentally; but of all of those employed the most important one, both as to quantity and effective results, has been "mustard gas" (dichlorethylsulphide). This was first used on a large scale at Ypres, July 12-13, 1917; and at times thousands of shells containing hundreds of tons of this gas have been fired in relatively short periods. Exact data as to the results can not at present be made public.

Dichlorethylsulphide (thiodiglycolchloride),



was first made by Victor Meyer<sup>1</sup> in 1886. He described it as a heavy oily fluid sinking below water and not miscible with it, of neutral reaction, having a faint, sweetish, ethereal odor but slightly suggestive of the sulphur compounds, and

\*From the Department of Pathology, University of Michigan, Ann Arbor, Mich.

with a boiling point of  $217^{\circ}$  C. He at once recognized the specific toxic properties of the substance from the fact that a laboratory worker engaged in making it, suffered from a severe skin eruption and a transitory conjunctivitis. As Meyer himself was not affected by exposure to the substance, he concluded that individual susceptibility to it must vary greatly. Animal experiments were carried out as follows: Two medium-sized rabbits were confined three to four hours in a closed cage ventilated by a moderately strong current of air passing through a glass tube containing strips of blotting paper saturated with thiodiglycolchloride. The animals became restless, rubbed their noses frequently with their feet. The nose and conjunctiva became reddened, and the eyes moist. Perspiration appeared to be increased. On the following day both eyes were severely inflamed, the lids glued together with purulent secretion. Marked snuffles developed, the lobes of the ears were much swollen, and the auditory passages showed a purulent inflammation. On the evening of the third day the animals died of a severe pneumonia diffused throughout both lungs. A strong rabbit that had inhaled the vapor of the substance for a few hours through a tracheal fistula, without exposure of the surface of the body to these vapors, died of a widespread pneumonia on the evening of the same day before other symptoms had appeared.

In rabbits in which the unbroken skin of the top of the ear had been touched with a fine brush containing a small amount of the chloride the site of the application showed no direct effects, but the entire ear became markedly swollen, and in one case a profuse purulent inflammation arose from the bottom of the auditory passage. In this case, the material could not have accidentally entered the auditory canal owing to the small amount used and the fact that it was applied to the outer surface of the ear. In one case in which the skin was scraped by the removal of the hair from the ear tip, the chloride applied by means of the brush caused an especially severe suppuration at this point, and also a marked swelling of the whole ear and inflammation of the eyes.

After subcutaneous injection of about two drops of the chloride into the back of a rabbit, there developed inflammation of both eyes, very severe snuffles, and on the third day death from pneumonia. At the point of injection no effects were apparent.

The experiments were discontinued because of similar unpleasant effects upon those engaged in the investigation. Meyer concluded that the most severe action of the chloride develops only after its entrance into the blood. Preliminary experiments with the glycol and the sulphide showed these to be non-poisonous.

In 1887, Meyer<sup>2</sup> stated that experimental animals surviving after application of thiodiglycolchloride (dichlorethylsulphide) to the ear, showed a persistent profuse suppuration and complete necrosis and falling off of the ear after a few weeks. Because of the marked toxic action of the dichlorethylsulphide, physiologic tests were made of the monochlorethylsulphide. This was found to possess poisonous properties like those of the dichlorethylsulphide, but less marked in intensity. In the comparison of these two substances and ethylsulphide, the chlorine-free sulphide was found to be harmless. The toxicity of

the mono- and dichlorethylsulphides, therefore, depends entirely upon the chlorine content.

From 1887 up to the last year, the poisonous nature of dichlorethylsulphide seems to have attracted little attention in either chemical or medical literature. Since its use as a poison gas in warfare, the name "mustard gas" has been almost universally applied to it because of the faint mustard-like odor; and from the war literature and war reports further knowledge of its toxic action has been gained.

The symptoms of mustard gassing are described in the British Army Reports as follows: Initial tendency to sneeze without irritation of eyes or lacrimation, with a gradually increasing nose and throat irritation, followed after about twelve hours by a free discharge of mucus from nostrils, painful irritation and inflammation of eyes, and occasionally vomiting. Many men had pain in forehead and stomach. Small blisters may form on face and neck; the skin between thighs is occasionally red, sore and sometimes blistered. Contact with fragments of shells and with the earth near shell holes may cause blistering through the clothes. None of these lesions manifests itself at once, they develop after several hours. The pain in the eyes may lessen after twenty-four hours, although acute inflammation may persist several days. In severe cases bronchitis and pneumonia may develop after thirty-six to forty-eight hours. There are usually no deaths under twenty-four hours. Prolonged exposure to small concentrations of the gas cause laryngitis and loss of voice, sufficient to put men out of action. Of the men affected practically all have conjunctivitis, about 95 per cent have throat and lungs affected, and about 70 per cent show skin burns. After six weeks the skin lesions are usually healed. The eye, pulmonary, and cardiac conditions recover more slowly.

Giraud,<sup>3</sup> in November, 1917, describes the early symptoms of mustard-gas poisoning, dividing them into three groups: eye lesions, respiratory tract lesions, and burns. All of these develop slowly, but the time of appearance varies for each lesion. The eye symptoms appear first, within six to twelve hours and are fully developed at the end of twenty-four to forty-eight hours. The respiratory affections usually appear at the end of two to four days. The skin lesions fall into two groups, the earlier burns, contemporaneous with the conjunctivitis or preceding it and later burns. The early burns are the most severe. The later burns appear at the end of four, five, eight, ten or even fifteen days. They are usually without danger. The three groups of lesions may be found singly or associated in the same individual. Giraud also finds that the eye lesions are most common, then follow those of the respiratory tract, and finally the burns.

*Eye Lesions.*—Conjunctivæ congested, vessels dilated, severe pain at times but usually moderate, moderate lacrimation, vision unimpaired, pupil reflexes normal, marked swelling of eyelids. The less severe cases heal in two to three days.

*Lesions of the Respiratory Tract.*—The initial symptom is aphonia which may come on suddenly or be preceded by a few paroxysms of coughing. It is usually complete, lasting three to four days. Tracheitis and bronchitis then

develop, the paroxysms of coughing are frequent and painful, closely simulating the cough of pertussis. At the end of two to three days, the patient discharges an abundant mucous or mucopurulent sputum. The affected patient can not breathe except in the erect position, and the majority decline to go to bed. Giraud has not observed hemoptysis. The respiratory affections are most intractable, and frequently relapse without any apparent reason.

*Skin Lesions.*—The early burns appear in twelve hours. These are the severe burns, presenting the appearance of large erythematous patches covered with large bullæ, containing a serous or seropurulent fluid. Occasionally, particularly on the scrotum, the large blisters are replaced by little purulent vesicles surrounding the roots of the hairs. *The pain is commonly quite severe*, and patients presenting burns of this degree are immediately evacuated. The lighter burns are more frequent, are insidious, appear slowly, but are the least serious of the mustard-gas lesions. Often they are unnoticed by the men, and are discovered only by chance. They appear as burns of the first degree in the form of erythematous plaques resembling sunburn. After a time, the central portion desquamates, leaving a slightly weeping surface without any actual blister formation. The burns appear most commonly around the joints of the lower extremities, thighs, genitals, buttocks, neck, and back. They are only exceptionally found upon the uncovered parts.

As to lesions of the digestive tract Giraud considers them exceptional, the frequent vomiting being attributable to efforts at coughing. He considers it probable, however, that lesions of the alimentary tract may be due to ingestion of food tainted by the gas.

All of the lesions observed by him have been purely local. He has only rarely seen signs of general disturbance, acceleration of pulse and fever, which he considers may have been due to other causes than the intoxication. The majority of the patients present an earthy pallor, but this is not surprising in men subjected to the fatigue of a prolonged stay in the first line trenches.

For prophylaxis and treatment he advises the use of a solution of bicarbonate of soda in a strength of thirty parts per thousand. Washing the eyes with the solution he found to be prophylactic against the conjunctivitis. For the burns he employs washing with the bicarbonate solution and a dressing of Vincent's powder (boric acid and calcium hypochlorite). The respiratory lesions he found resistant to treatment, which has been wholly symptomatic.

Teulières,<sup>1</sup> in the same month, November, 1918, describes the action of the gases used in warfare upon the visual apparatus. Under the heading of the "new gas," which he states is composed of carbon tetrachloride and ammonium-bichlorosulphide,\* but described by the majority of soldiers as having a mustard odor, colorless, very heavy and impregnating the earth, and which, therefore must have been dichlorethylsulphide, he says that the ocular lesions produced by it range from simple palpebro-conjunctival reddening up to the most severe burns of the conjunctiva and cornea. Since the gas seems to operate only in the presence of water, he advises against bathing the wounded. The treatment he advises is purely symptomatic.

\*Corrected by author in erratum.



Mandel and Gibson<sup>5</sup> give a brief account of the clinical manifestations and treatment of mustard-gas poisoning, adding nothing to what has been gained from Giraud's article, except the statement that the myocardium, as demonstrated by necropsy, is not damaged. Their summary is as follows:

1. An interval (four to sixteen hours) of freedom from distress exists between the actual gassing and the onset of the symptoms.
2. The cardinal symptoms, conjunctivitis, laryngitis, bronchitis, and skin burns, are all due to the excoriating effect of the gas.
3. The principal complications are early pulmonary edema and relatively late bronchopneumonia.
4. Dry clothing and sleeping quarters may prevent the development of symptoms after slight exposure, and possibly may lessen the severity in those more severely gassed.

#### EXPERIMENTAL

Haldane<sup>6</sup> states that he has confirmed Meyer's statement that subcutaneous injection of the substance is capable of causing conjunctivitis and death from pneumonia, owing to the absorption of the gas into the circulation.

Kolls and Gilbert<sup>7</sup> report that 10 mg. of an alcoholic solution injected into the muscles and intravenously produced nothing more than temporary inactivity and loss of appetite, with recovery in one week; 50-100 mg. in alcohol and water emulsion produce convulsions within an hour and death in three hours. *Mice* exposed to the vapor after five minutes became depressed, developed irritation of nostrils and marked dyspnea. Except when high concentrations were used death was delayed two to six days. *Cats* exposed fifteen minutes to a concentration of 1:2000 showed lacrimation and restlessness; after two hours cat became wild, kept eyes closed; after twenty hours became dyspneic and died at thirty-six hours. A fifteen-minute exposure to a concentration of 1:10,000 gave similar results; the animal died in four days of pneumonia. In twenty hours photophobia and purulent conjunctivitis developed. *Rabbits* exposed for four hours to less than 1:100,000 concentration closed their eyes towards the end, developed well-marked conjunctivitis at end of twelve hours, and died in two days. After exposure to 1:20,000 concentration conjunctivitis developed in six hours, and was marked in twenty-four; there was marked dyspnea and snuffles after twenty hours.

Raper and Ball<sup>8</sup> found that rabbits exposed for five minutes to a concentration of 1:20,000 developed a very slight irritation of the eyes after five minutes; six hours later both eyes were kept closed, and there was a definite conjunctivitis which grew steadily worse with signs of nasal catarrh. When killed after four days there was found an intense congestion of the trachea and some patches of bronchopneumonia. A fifteen-minute exposure caused marked discomfort after ten minutes in the form of a marked irritation of eyes and nose; five hours later conjunctivitis and snuffles had developed. This was worse the following day, and the inflammation of the eyes became purulent. When killed on the fourth day similar changes to the above were found. Thirty-minute exposures produced the same results.

Marshall and Miller<sup>9</sup> worked with dogs. They found that higher concentrations (38 mg. per liter) caused immediate excitement, irritation of eyes and lachrimation, followed by dulling of cornea and depression. Frequent retching and vomiting was observed. At the end of exposure or shortly afterwards the mucous membranes were inflamed, and, in some cases, the skin on face and bare parts of hind legs became red. There was continued retching and vomiting, nasal discharge and salivation, dyspnea, tracheal rattle, and death in four to ten hours. With lower concentrations there was practically no sign during exposure. In twenty-four hours there developed conjunctivitis, nasal discharge, and tracheal rattle, and animals became depressed. There was no vomiting, as a rule. Death occurred in twenty hours to twelve days.

Kolls and Gilbert<sup>10</sup> showed that 1:100 alcoholic solutions act as skin irritants. With increasing dilutions up to 1:1000 the skin is killed, but there is little difficulty in healing. Further dilution produced no irritation. The skin lesions were found to show great individual variations.

Test tubes containing air saturated with mustard-gas vapor applied to the skin cause erythema in twenty-four hours after five minutes' exposure; after ten minutes' exposure erythema was more marked; after twenty minutes' exposure there developed in twenty-four hours a red papular area just short of a blister, with hyperesthesia. When the concentrated vapor is allowed to act on the skin for a short time a vesicle may not be produced, but only a red edematous patch with slight soreness on surface, something like a sunburn (Royal Society War Commission Reports).

One to two drops of a 1:100 solution in alcohol applied to the arm caused varying degrees of itching and blistering. Complete healing took place in seven to eight days, although a scar remained.

Other experimenters<sup>11</sup> find mustard gas to be a blistering agent of great power, producing erythema and blistering the skin as well as causing acute conjunctivitis, tracheitis and bronchitis. The intensity of the action seems to be increased by humidity, although if hands are washed immediately after exposure no lesions are produced. If the skin is touched with a glass rod just moistened with the gas and then wiped with cotton there results in two hours a red patch larger than the area touched. This increases in size, and at the end of four hours may be as large as a sixpence. After twenty to thirty hours a well-marked tense vesicle develops, with erythematous area around vesicle. If the latter is opened under aseptic precautions and infection is avoided, the lesion heals normally, but redness persists for three weeks or more. The whole process is painless.

Subjects exposed in chambers containing 1:10,000 (0.709 grams per M<sup>3</sup>) of impure oil, when wearing French respirators, showed after six hours irritation of the skin, vomited and felt ill. On the next day there was marked erythema of the scalp, chest, inside of arms, hands, thighs and genitals. The erythema was more marked on one forearm that had been smeared with vaseline and lanolin. On the arm and on the genitals large blisters were produced.

*Summary.*—The general effects without protection in animals and in man were found to be conjunctivitis, sore throat, hoarseness, gradual necrosis of mucous membrane of air passages, bronchitis, later purulent in character, lead-

ing to bronchopneumonia, which may terminate in death, and more or less widespread burns of skin. These affect chiefly covered parts, but the face and hands may also show burns. The skin lesions develop as follows: Erythema, with some irritation or no pain, followed by blister, accompanied, particularly on the genitals, with much swelling and edema. The erythema is attended by diapedesis of red cells. Even when no blisters are formed, there may be dark purplish patches, the color of which does not disappear on pressure. Later these become pigmented dark-brown.

#### PATHOLOGY

Mackenzie<sup>12</sup> states the most important pathologic changes to be those found in the eyes, skin and respiratory system. In experimental animals there is diffuse clouding of the cornea at once which goes on to exfoliation of the corneal epithelium and to ulceration. The corneal vessels show a marked acute congestion; there is an abundant serous discharge, which later becomes purulent. The skin, where there is no hair, about the lips, nose, genitals, nipples and between toes, shows bright red discolorations.

The upper air passages show patches of intense hemorrhagic redness, in the mouth sometimes to the extent of ulceration, particularly at points of contact with teeth and cheeks. The pharynx, epiglottis and larynx are reddened. From the upper end of the trachea throughout the entire bronchial tree, the mucous membrane is covered with a thick translucent membrane of a pale yellowish color, which is easily pulled off in large strips. It extends down into the smallest bronchi, forming everywhere a complete lining to the tubes. There is no free fluid or froth in trachea or bronchi, as is constant in other forms of gassing. In the medium and small bronchi the membrane occludes the lumen, causing patches of atelectasis. These may be only 0.5 to 1 cm. in diameter, or may involve a whole lobe, depending upon the size of the occluded bronchi. Such atelectatic areas are dark reddish purple, depressed and nearly airless. Their relation to the plugged bronchi can be easily determined. No pleural effusion noted in animals.

No constant changes were found in other organs. Focal necroses were noted in the liver. These appeared on the surface and on cut section as gray spots, size of pinhead or smaller with a central red point. In three animals punctate hemorrhages were found in the adrenal cortex. In about half of the animals swelling and hyperemia of the mucosa of stomach and duodenum was noted; in one case circumscribed hemorrhagic ulcerations were found near the pylorus. No positive relationship of these findings to the gassing was demonstrated.

Haldane<sup>13</sup> noted the pathologic changes in two cats, one exposed two hours and killed four days afterwards; and the other exposed one and one-half hours and killed nine days after. The first cat showed desquamation of tracheal mucosa and the formation of a plug of leucocytes and red blood cells. Similar lesions, but more severe, were found in the bronchial tree; and the lungs showed leucocyte exudation into alveoli; edema and desquamation of alveolar epithelium, and abscesses. The second cat showed thickening and collapse of the alveolar walls,

congestion of the pulmonary vessels, desquamation of nasal epithelium, and inflammation of the skin of the ears.

McNee<sup>14</sup> gives autopsy summary of fatal cases. The points noted were acute inflammation of air passages, desquamation of mucous membrane, and formation of false membrane. To this, infection is added in the form of acute purulent bronchitis with atelectasis and bronchopneumonia. Acute emphysema may be present also. Superficial burns are a prominent feature. Acute hemorrhagic nephritis and edema of lower limbs were noted. In two cases degenera-



Fig. 1.—One hour after application of mustard gas. Stage of crythema.



Fig. 2.—Three hours after application. Widening and deepening of the erythematous zone with well-marked edema. Development of secondary paler areola.

tive changes in the central portion of liver lobules and minute hemorrhages in brain were noted.

McLeod<sup>15</sup> from seven autopsies on men killed in action by the gas, concludes that the danger to life from secondary infection is greater than the toxic action of the gas itself.

Dunn<sup>16</sup> from four cases states that the chief lesions are: 1, severe and last-



ing damage to bronchi and bronchioles; 2, persistence of edema; 3, severe infection of respiratory tract, bronchopneumonia, and in two cases hemorrhagic exudations.

*Summary.*—The material given above represents the entire pathologic knowledge of mustard gassing available in the literature at the present time. As is readily seen, this pathology is very superficial and incomplete, being made up almost entirely of rather loose superficial gross descriptions. No detailed gross pathologic observations exist at present; and there is practically no microscopic



Fig. 3.—Eighteen hours after application. Formation of vesicle on an erythematous base.



Fig. 4.—Twenty-two hours after application. Vesicle 4 mm. high, tense, fluid content slightly opalescent. Floor of vesicle appears yellowish white, necrotic.

pathology. If a histologic study of the lesions produced by mustard gas exists in the literature, we have not been able to find it. For this reason, as soon as we heard of the new gas last summer, our laboratory made efforts to secure the substance for the purpose of experimental pathologic work with the view of determining the exact nature of the tissue lesions. Through the kindness of Professor Moses Gomberg, we were furnished with an ample supply of pure mustard gas, and more recently through the courtesy of Doctor Wm. Hale and

the Dow Chemical Company of Midland, Michigan, with an impure form of the gas. The great majority of our experiments have been carried out with the pure dichlorethylsulphide furnished by Doctor Gomberg. This is a clear, colorless, heavy, oily liquid having a boiling point of  $217^{\circ}$  C. and possessing a faint mustard odor.

The present investigation concerns itself solely with the study of the gross and microscopic pathology of the cutaneous lesions, produced in man and animals by the direct application of the liquid. The human material was obtained from



Fig. 5.—Forty-six hours after application. Collapse of vesicle with nearly complete absorption of fluid. Epidermis in fine wrinkles and folds, and of a yellowish brown tint.



Fig. 6.—Seventy-two hours after application. Beginning eschar formation. Central area bright yellowish brown in color, surrounded by a whitish zone 1.2 mm. in width.

auto-application, amputation material with consent of the patient, and accidental chemical laboratory lesions. Through auto-application we have been able to study a complete series of lesions through all stages from inception to healing.

#### ACTION OF DICHLORETHYLSULPHIDE ON HUMAN SKIN

The dichlorethylsulphide was applied by means of a capillary pipette in uniform droplets estimated to be about .002 c.c. When applied to the skin this drop

at once spreads out over an area 3 to 4 mm. in diameter and is completely volatilized, or at least disappears, in one to two minutes, according to atmospheric conditions.

Following is a protocol of the most important stages in the development of the skin lesion produced in this manner. We have found that the rate of production of the lesion and the intensity of the reaction vary over a considerable range in different individuals, notably in a Charcot-joint leg and in a case of malignant disease the development of the vesicle was delayed.



Fig. 7.—Four days after application. Beginning sloughing. Loss of necrotic wrinkled epidermis. Persistence of white zone and zone of erythema.



Fig. 8.—Nine days after application. Beginning separation of necrotic base from the peripheral white zone. More marked erythematous zone.

*Auto-application to skin of normal individual.* March 12, 1918, 2:30 p.m. Standard droplet applied to flexor surface of left forearm. In one minute the liquid completely disappeared giving off a strong mustard odor. There were no subjective symptoms. In about ten minutes there appeared a delicate silvery gray sheen over the surface of the area of application. This was soon followed by a faint flush which gradually deepened and spread until it was about 7 mm. across. Photograph in Fig. 1 was taken one hour after application. At this stage the erythema was influenced by changes of temperature, etc., alternately paling and reddening. Whenever the area became somewhat paler the superficial silvery luster was visible. During the second and third hour a well-marked edema appeared and the erythematous zone became wider and more deeply colored.

Same day, 5:30 P.M. At this time the lesion measured 15 mm. in diameter. It was slightly elevated and had a marginal zone somewhat deeper red than the central portion. Outside of the red zone there was a very faint, barely perceptible zone showing less color than the remainder of the skin. (Fig. 2, photograph three hours after application.) In the next several hours there was no change.

March 13, 1918, 6:30 A.M. Sixteen hours after the application a vesicle began to form. At 8:30 A.M., eighteen hours after the application this was at its height. (Fig. 3.) At this time the lesion measured 25 mm. in diameter. It presented an erythematous base, slightly elevated, and fading gradually into the surrounding skin. Upon the summit of this erythematous base, there rose a tense vesicle 6x9 mm. in area and 3 mm. high. This was filled with a clear pale yellow fluid. Up to this time there had been no subjective



Fig. 9.—Eighteen days after application. Raising and separation of the yellowish brown crust. Beginning fading of the erythematous area leaving a pigmented border.



Fig. 10.—Nineteen days after application. Base of lesion after complete separation of the heavy crust of the eschar. Granulation tissue base.

symptoms, but with the formation of the vesicle there was slight smarting, increased by pressure.

Same day, 12:30 P.M. Twenty-two hours after application. (Fig. 4.) At this time the vesicle covered no greater area but was 4 mm. high and still very tense. Its fluid content had become slightly cloudy or opalescent. With a hand lens the base of vesicle can be seen through the fluid content and appears yellowish white, opaque and necrotic. Around the border of the inflamed base of the vesicle there is a definite secondary areola.

March 14, 1918, 12:30 P.M. Forty-six hours after application. (Fig. 5.) By this time there was nearly complete absorption of the fluid from the vesicle. The epidermal



covering of the vesicle is thrown into fine wrinkles and folds and has taken on a yellowish brown tint. The zone immediately around the base of the vesicle is now pale pinkish white and about 1 mm. in width. Outside of this the flushed areola persists. The total width of the lesion is now but 17 mm.

March 15, 1918, 2:30 p.m. Seventy-two hours after application. (Fig. 6.) The central portion of the lesion measures 8x4 mm. and is of a bright yellowish brown color. Around this is a white zone 1 to 2 mm. in width. Outside of this is a zone of erythema most marked about the base of the collapsed vesicle and fading peripherally. The total width of the lesion is now 18 mm.

During the night of March 15 to March 16 the delicate wrinkled epidermis was rubbed off, leaving an excavated area with a grayish yellow-white moist base. The excavated area measured 6x4 mm. The border was somewhat irregular and slightly over-



Fig. 11.—Forty-nine days after application. Scar with brown pigmented areola. Slight puckering of scar.

hanging. The white zone, about 1 mm. in width, still persists at the border and the erythematous zone outside of this is about the same width as before. The base became slightly glossy upon drying. Fig. 7 was taken at 5:30 p.m., March 16, 1918.

March 17, 1918, 9:00 p.m. Total excavated area somewhat diminished. Border smoother. Floor not quite so deep. Total width of lesion is now 15 mm.

March 21, 1918, 4:00 p.m. A brownish crust representing the necrotic base is beginning to loosen at the edges where it is white and slightly desquamating. About this is a marked erythematous areola, 4 mm. wide. (Fig. 8.)

March 22, 1918, 9:00 a.m. During the night there was marked itching, the only pro-

nonounced subjective symptom so far noted. The crust loosened entirely and came off. Beneath the crust there was a small amount of thin purulent fluid. The erythematous zone is less marked. The base of the excavated area is again dry and covered with a yellowish brown crust. The margin of the excavation is whitish with edges slightly puckered. The central portion of the lesion measures 5x9 mm.

March 30, 1918. Since March 22, the area of excavation has gradually become covered with a yellowish brown crust which has now become elevated nearly 2 mm. above the surrounding surface. The zone of erythema is fading, leaving a yellowish-brown pigmentation. The inner portion of this zone is shiny and somewhat puckered and there are a few minute desquamating scales at the border of the dense crust. The entire width of the lesion including the zone of pigmentation is 3 cm., the reddened, somewhat shiny



Fig. 12.—Typical "mustard gas" vesicle. About twenty-two hours after application. Photograph somewhat enlarged to show details of the vesicle.

zone, is 2 to 3 mm. in width, the scaly desquamation about 1 mm., and the elevated crust 4x7 mm. (Fig. 9.)

April 1, 1918. The dark crust or scab became loose and came away leaving a white dry slightly granular area nearly flush with the surface of the skin. This measured 4x8 mm. (Fig. 10.)

May 1, 1918. Healing is now nearly complete, the lesion consisting of a thin scar, pinkish white in the central portion and whiter, more opaque, at the margin, with very slight puckering. Around this is a brown pigmented areola. The whole area, however, is redder than normal skin. (Fig. 11.)

## MICROSCOPIC APPEARANCES

The changes in human skin were studied microscopically from one-half hour up to thirty-six hours, including the development of the vesicle and beginning eschar formation. As the lesions at one-half hour, eighteen hours, and thirty-six hours represent three distinct stages, these will be described in detail.

*Lesion One-half Hour after Application*

*Epidermis.*—The horny layer is relatively thicker than normal and split up into flat scales and layers, loosening readily from the stratum lucidum. The stratum lucidum has a slight brownish color. The granular layer is flattened; the cells drawn out parallel to the surface; the nuclei are pyknotic. The stratum germinativum is markedly shrunken, in many places only one-third to one-half



Fig. 13.—Mustard-gas burns of both hands, six days after exposure, in a chemical laboratory assistant.

as wide as normal, its nuclei pyknotic and the cytoplasm shrunken about the nuclei. Occasional vacuoles are found in the lowest layers, but the most marked change is the shrunken appearance of the whole epidermis, both cytoplasm and nuclei. At the border of the lesion the epidermis passes gradually into the normal condition.

*Papillary Layer of Corium.*—In the central part of the lesion, the capillaries are contracted and contain but little blood. In scattered capillaries the red blood cells are agglutinated and stain with eosin as bright red hyaline masses. Such agglutination thrombi, however, are not a common feature of the picture and in the larger vessels thrombosis does not occur. The endothelium of the capillaries of the papillary layer shows marked pyknosis, karyorrhexis and disintegration of the nuclear chromatin. Chromatin dust is found around many of these capil-

laries and also many of the connective tissue cells of the upper portion of the papillary layer show marked karyorrhexis. The cytoplasm of many of the endothelial cells of the capillaries is vacuolated, showing hydropic degeneration or edema. About these capillaries there is a clear space due to a perivascular edema. Around some capillaries this is very marked. Many of the capillaries show diapedesis of leucocytes along their course (Fig. 15), but in the central



Fig. 14.—Scar on back of hand six months after accidental burn with a dilute solution of dichloroethylsulphide dropped upon back of hand and immediately washed away. Chemist. Burn exhibited stages of vesicle and eschar formation similar to those described in the above protocol, followed by very slow healing.

part of the lesion there is practically no hemorrhage and the vessels are conspicuous for their contraction and anemia.

*Corium Proper.*—The vessels running through the corium show similar changes in their endothelium but there is little leucocytosis or white cell migration. The larger vessels contain more blood. No thromboses or hemorrhages are present. The lymphatics are dilated and the nerve trunks show karyorrhectic nuclei and edema.





Fig. 15.—Human skin one-half hour after application of mustard gas. Shrinking and pyknosis of epidermis with desquamation of horny layer. Capillary changes as described in the text, including well-marked migration of leucocytes.

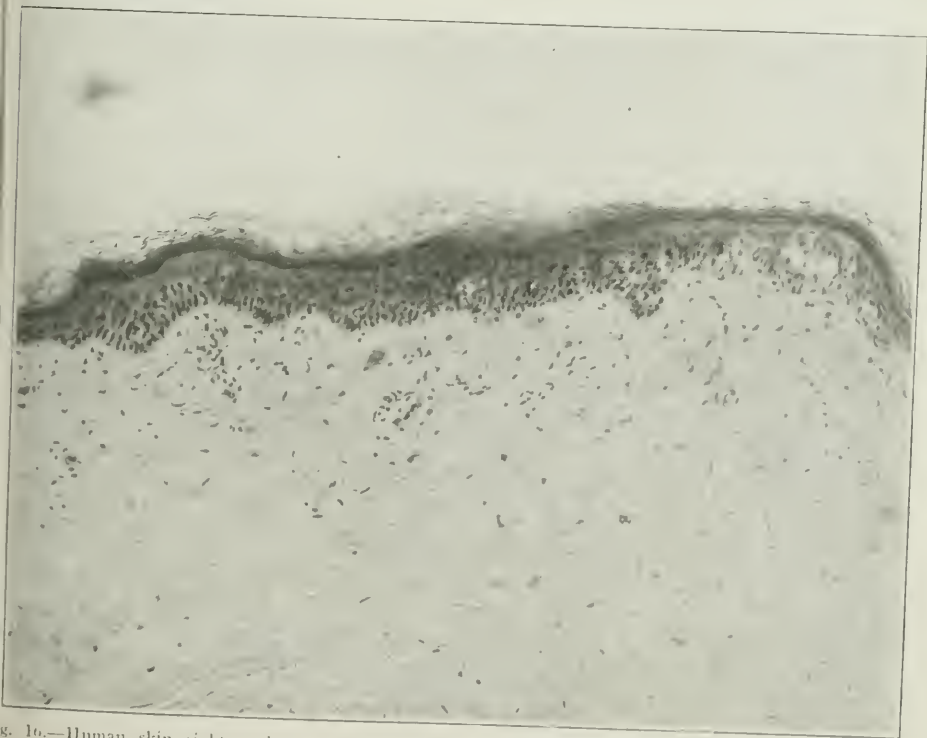


Fig. 16.—Human skin eighteen hours after application. Transition between slightly damaged epithelium and epithelium showing hydropic degeneration. Early blister formation.

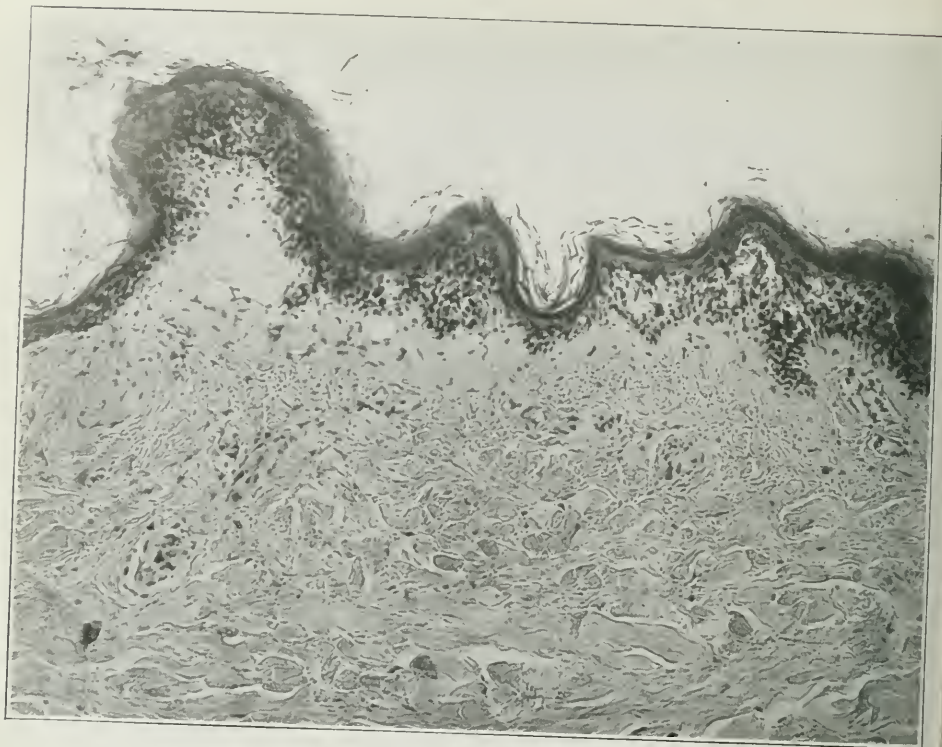


Fig. 17.—Human skin eighteen hours after application. Early vesicle formation.

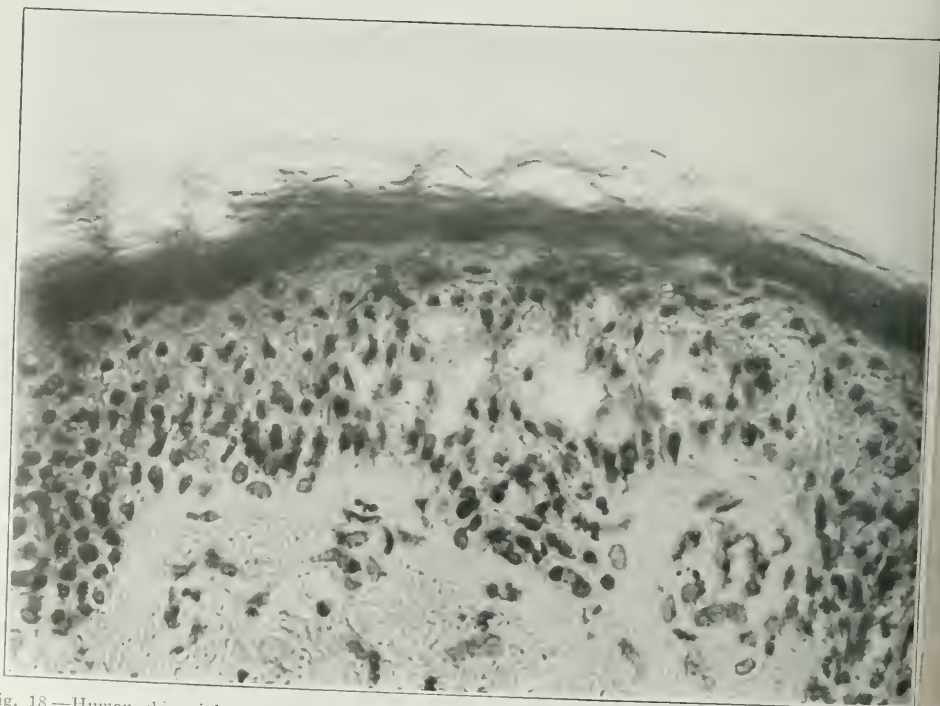


Fig. 18.—Human skin eighteen hours after application. High power view of hydropic change with early vesicle formation.

*Hair Follicles.*—Along the hair follicles the squamous epithelium shows changes similar to those of the surface and the capillaries about the hair follicles also show changes similar to those described above. In the neighborhood of the hair follicles the corium is affected more deeply than elsewhere, showing a distinct penetration through the hair follicles.

*Sweat Glands.*—The epithelium of the sweat glands shows no apparent changes, although the vessels about them show changes similar to those described above.

*Sebaceous Glands.*—Shrinking and pyknosis of cells similar to that seen on the surface.

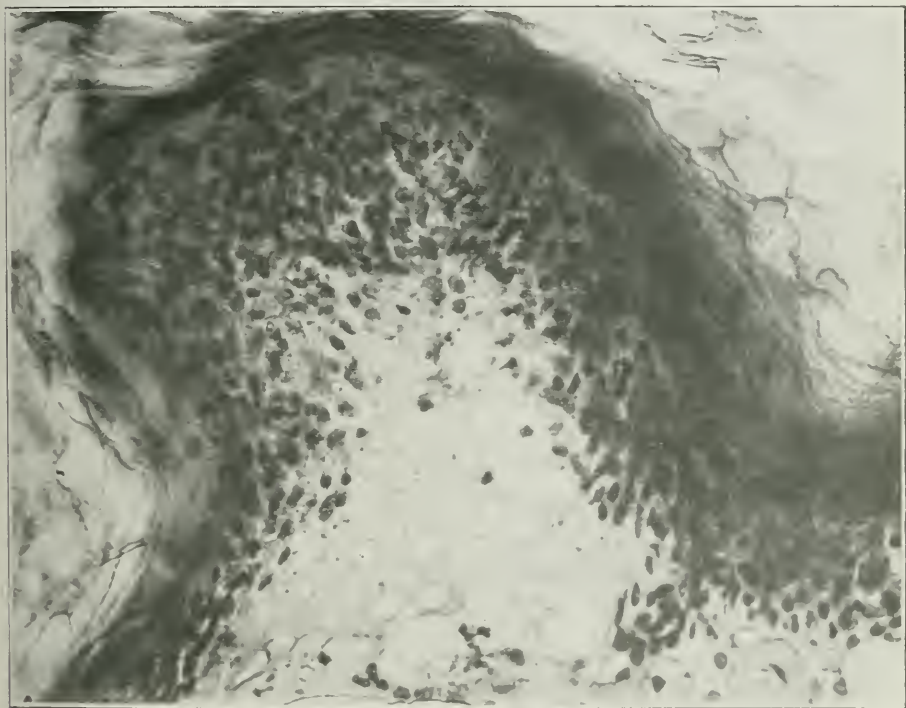


Fig. 19.—Human skin eighteen hours after application. High power view of small vesicle. Separation of epidermis from papillary layer.

In the transition border there are small hemorrhages by diapedesis.

*Lesion Eighteen Hours after Application.* (Figs. 16, 17, 18, and 19.)

*Epidermis.*—In the central part of the lesion there is a marked liquefaction and hydropic change in the cytoplasm of the epithelium. This varies greatly in degree. The horny layer is in part desquamated and loosened, the stratum lucidum is widened and more dense than normal and stains brownish red. Over many of the papillae small vesicles have already formed, the majority of the epithelial cells having undergone liquefaction. In some places the epidermis is lifted from the papillae by the collection of fluid beneath it. The liquefaction of the cell cytoplasm extends deep down into the hair follicles and into the sebaceous



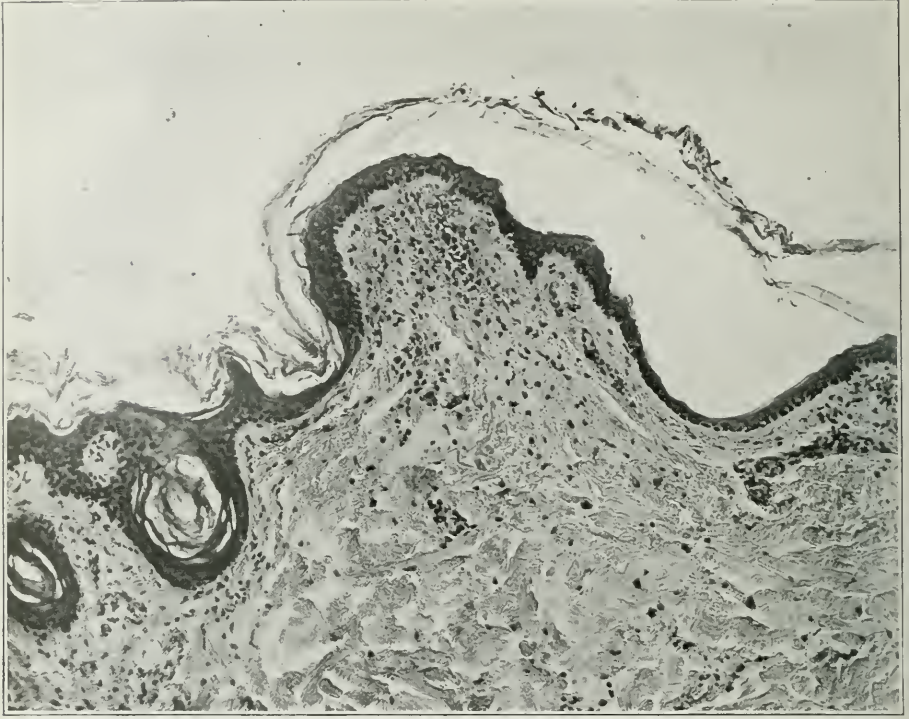


Fig. 20.—Human skin, thirty-six hours after application. Vesicle formation in epidermis and leucocyte infiltration of papille.

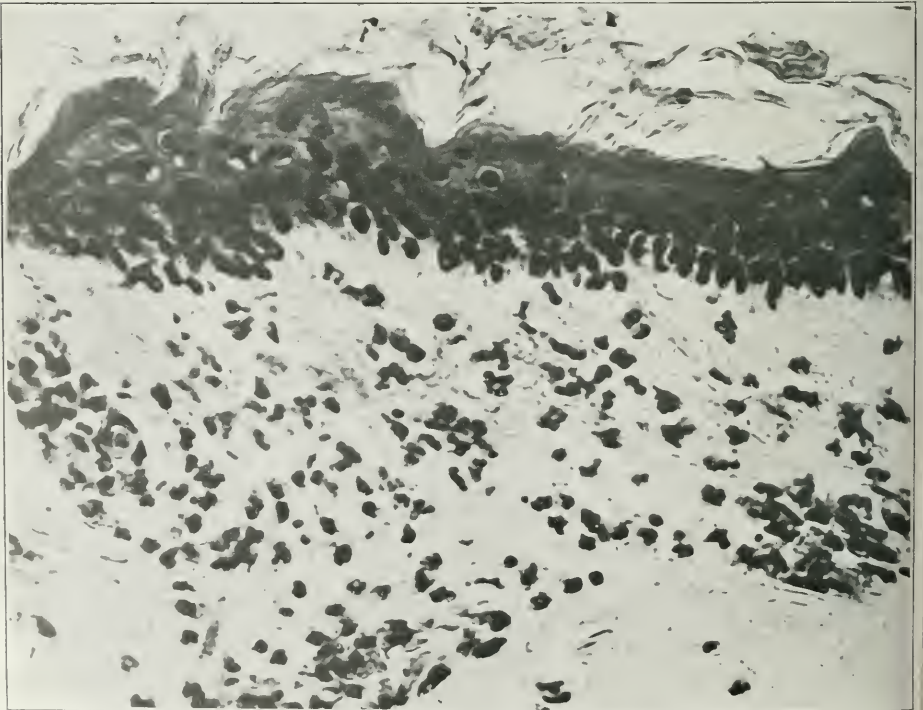


Fig. 21.—Human skin thirty-six hours after application. High power view at border of lesion showing changes in epidermis and leucocyte infiltration and edema of papillary layer.



glands. The stratum germinativum has lost its continuity in many places and the cells are completely necrotic.

*Papillary Layer of the Corium.*—The connective tissue is edematous, stains bluish, and contains many degenerating nuclei. There is an increase in the number of wandering cells and many of these show karyorrhexis. Around all the capillaries there is a zone of edema and small-celled infiltration. Small hemorrhages by diapedesis are scattered through the papillary layer and upper portion of corium, particularly around the hair follicles.

*Corium Proper.*—The blood vessels contain more blood than in the earlier lesion, particularly the deeper ones. The larger ones show a marked congestion and the lymphatics are dilated with a lymph rich in albumin. Around the hair follicles the edema, liquefactive changes, and hemorrhages are more marked than elsewhere. The sweat glands show a marked edema of the interstitial connective tissue, congestion of the capillaries, leucocyte infiltration and small hemorrhages by diapedesis. Some of the glands show a marked necrosis of the epithelium but these changes vary greatly in degree.

*Subcutaneous Tissues.*—The vessels are congested, lymphatics dilated and there is edema of the adipose tissue. The vascular changes extend along the smaller capillaries even into the subcutaneous tissue.

#### *Lesion Thirty-six Hours after Application (Figs. 20 and 21)*

*Epidermis.*—Horny layer more compact but ragged, in many places infiltrated with leucocytes. Epidermis nearly completely necrosed, in some places lifted from the papillary layer. The remaining nuclei are markedly pyknotic or fragmented. In many areas only the lowest layer of nuclei persists. There are also collections of fluid between the horny layer and the portions of rete remaining.

*Papillary Layer.*—The papillary layer shows marked edema, the capillaries are congested and there are many hemorrhages by diapedesis. The entire papillary layer is infiltrated with leucocytes, many of which show karyorrhexis.

*Corium.*—There is a leucocyte infiltration throughout the entire corium but less marked than in the papillary layer. It is most marked around the hair follicles and around the sebaceous glands and sweat glands. The congestion and edema are also most marked around these structures. Some of the smaller vessels show marked necrosis of the wall with leucocyte infiltration and diapedesis. Scattered areas of edema and small celled infiltration extend even into the subcutaneous tissue where the vessels are markedly congested.

#### *Later Stages in Human Skin*

Inasmuch as the later stages in human skin parallel those in the lower animals and as the specific differences between the action of mustard gas on human skin and on the skin of the rabbit, guinea pig and cat exist only in the early stages, it seems necessary here to omit a more detailed description of these changes and to summarize them as follows:

1. About forty to fifty hours after application collapse of vesicles and progressive necrosis.

2. About seventy-two hours after application progressive necrosis and beginning eschar formation.

3. Four to six days after application, necrosis complete, beginning separation of slough. Edema and hyperemia persistent.

4. By the nineteenth day, complete separation of slough. Slow healing and scar formation.

5. For an indefinite period, congestion and pigmentation.

All of these descriptions applied to the effect of a standard drop of pure mustard gas in the absence of infection. The course will naturally vary with the concentration, amount, time, etc.

#### PREVENTION OF THE LESION

A preliminary report is made here of methods tried out to prevent or abort the lesion after the application of mustard gas. We still regard this work as in the experimental stage and the statements made here only tentative. In some of the English and French reports great emphasis is laid on the fact that water greatly increases the intensity of the lesion. Our experience shows this to be unfounded. If the application of mustard gas to the skin is followed by thorough washing within a few minutes, the resulting lesion is far less intense though usually spread over a larger area. If this can be done before the expiration of two minutes, no necrosis and no vesicle formation results and there is only a hyperemia and a more or less transient edema. Washing immediately with tincture of green soap absolutely prevents the development of a lesion and if this is done two minutes after application, just as the droplet has completely volatilized, only a slight but persistent hyperemia results. This method of prevention, while it might not be applicable under the conditions of warfare, would apply to prevention in factories, chemical laboratories, and munition depots. Bleaching powder, sulphur chloride, and sodium bicarbonate are also of value but from our present results we would recommend immediate washing with tincture of green soap.

#### ANIMAL EXPERIMENTS

The rabbit, guinea pig, and cat were employed for these experiments. The skin of the belly was shaved and standard drops were applied after the irritation from shaving had subsided. The character of the tissue lesion and the reaction in these animals, proved, in the early stages, to be essentially different from the lesions in human skin. For the purpose of brevity and conciseness the protocols are condensed as below.

*Rabbit.*—Within two hours after application of the standard drop there develops a very marked edema, much larger than the area touched by the drop of mustard gas. This edema is subcutaneous, appearing as a definite tumor mass rather sharply circumscribed, over which the cuticle can be moved. The surface of the area appears gray and cloudy, the skin losing its normal translucency and appearing as if cooked. In some cases the blanching appears to extend into the deeper portion of the skin. About this gray area there is but slight hyperemia

By the third day after the application, the epidermis over the area undergoes complete necrosis and there is formed a slough without any vesicle formation. Vesicles were never observed in the rabbit. This slough is held on apparently by the hairs. It gradually is elevated, separates, and contracts, and may not be cast off for three or four weeks. When shed, the lesion below is practically healed. The most striking thing is the marked edema at the beginning, the per-



Fig. 22.—Rabbit. Application of mustard gas at 11:30 A.M. Droplet used was slightly larger than the standard. Marked subcutaneous edema as seen at 4:00 P.M. on the same day.

istence of this edema without vesicle formation, and the slow healing in the absence of infection.

Various protective experiments were tried out, a number of substances being used to prevent the lesion or lessen its severity—washing with water, soap and water, lead acetate, lead acetate and silver nitrate, zinc oxide ointment, zinc oxide paste, bleaching powder, sodium sulphide, tincture of green soap, potassium permanganate. The application of most of these substances five minutes

after the application of the mustard gas lessens the edema and renders the lesion more diffuse but does not prevent necrosis. The use of potassium permanganate resulted in even greater edema than the untreated control while strong sodium sulphide solution was found to be disadvantageous because of the necrosis produced. (Figs. 22, 23, and 24.)



Fig. 23.—Rabbit. Skin of belly shows results of four applications of standard drops of mustard gas. Above two areas, of typical edema, the one on the rabbit's right untreated, the one on the left washed off in five minutes by water. The latter is more diffuse, larger in area, but less intense. Below on the rabbit's right, an area washed after five minutes with soap containing an excess of free alkali. This area shows the least reaction. On the lower left is an area treated after five minutes with potassium permanganate. The reaction here is the most marked.

*Guinea Pig.*—Practically identical results were obtained in the guinea pig with the standard drop of pure mustard gas; namely, within a few hours marked subcutaneous edema followed in a few hours by necrosis of epidermis and papillary layer without vesicle formation and exhibiting very slow healing.

*Cat.*—The same results were obtained as for rabbits and guinea pigs. Sub



cutaneous edema without vesicle formation, followed by necrosis and slow healing.

#### MICROSCOPIC PATHOLOGY OF ANIMAL LESIONS

The changes observed in the development of the lesions in the rabbit, guinea pig, and cat are essentially the same and are summarized here as follows:

1. *Stage of Marked Edema.*—The most striking feature of this stage is the

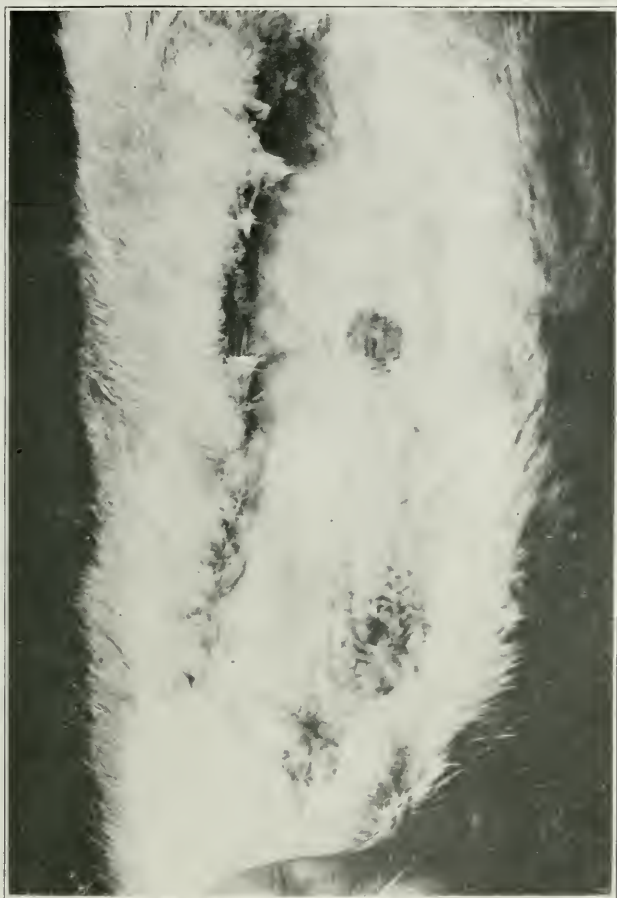


Fig. 24.—Rabbit. Two areas of mustard-gas application. Advanced eschar formation.

intense edema which is sharply localized to the subcutaneous tissue and fascia, but extends into the muscle through the abdominal wall. The connective tissue fibrillae are widely separated and the tissue spaces are filled with a heavy albuminous precipitate staining deep pink with eosin. The muscle fibers of the abdominal wall are separated, and even in two hours there is a leucocytic infiltration into the muscle. The edema extends 0.5 to 1 cm. below the epidermis. The epidermis is shrunken, cells pyknotic and in the central portion of the lesion completely necrotic. In the upper layer of the corium numerous degenerating nuclei are seen. The blood vessels show degeneration of the endothelium with small

hemorrhages by diapedesis and leucocyte migration. Around each vessel there is an area of edema. There is, however, no vesicle formation in or beneath the epidermis as in the human cases. The changes are more uniformly diffuse in the animal than in the human skin and the depth of penetration greater. The



Fig. 25.—Rabbit. Low power view of mustard-gas lesion in rabbit two hours after application. Extreme subcutaneous edema. Epidermis but slightly changed.



Fig. 26.—Guinea pig. Low power view of mustard-gas lesion five and one-half hours after application. Extreme subcutaneous edema. Epidermis necrosed in center of lesion.

localized penetration along the hair follicles, so prominent in the human skin does not show in the animal skin, the greater number of hair follicles permitting a more uniform access of the liquid. There are no thromboses in the damaged



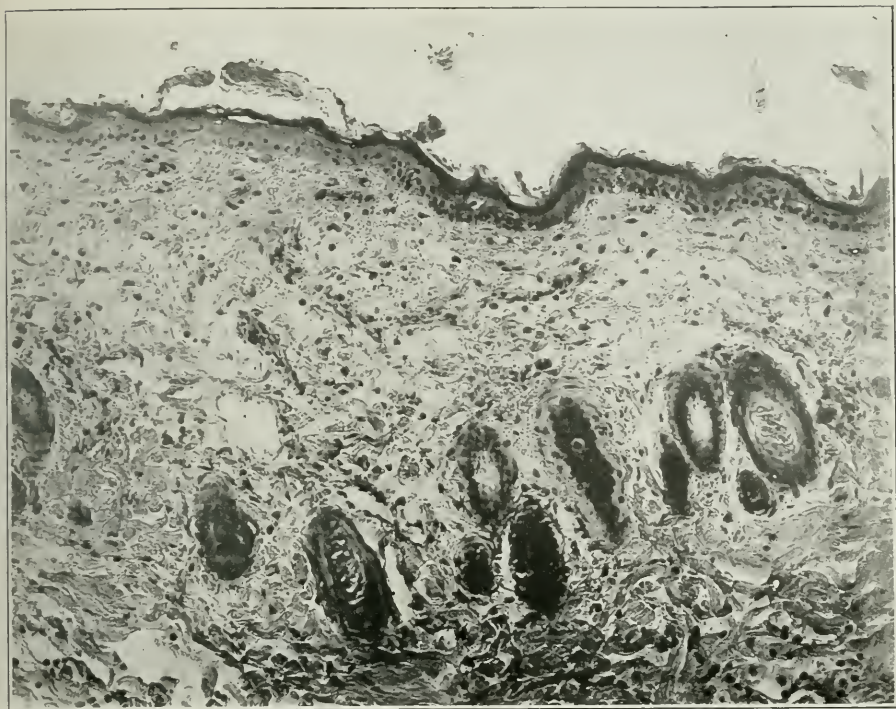


Fig. 27.—Rabbit. Border of lesion two hours after application. To the right of the middle the epidermis is still living, to the left nearly completely necrosed, necrosis extending into the upper portion of the corium. Early edema.



Fig. 28.—Rabbit. Two hours after application. Changes in epidermis and corium. Marked vascular change with beginning migration of leucocytes. Small hemorrhages by diapedesis. Early edema.

area. The hemorrhages are relatively small and, as in the human skin, the vessels in the immediate lesion are contracted and anemic. Around the borders of the lesion they show marked congestion.

2. *Stage of Necrosis.*—The necrosis of the epidermis and of the underlying tissues steadily becomes more prominent because of the loss of nuclei in the epidermis and upper part of the corium of the central part of the lesion. By the fifth and sixth day after the application of the mustard gas the edema has subsided to a marked degree and the central part of the lesion may be entirely without nuclei as far as the lower portion of the corium. It is bloodless, rather dry,

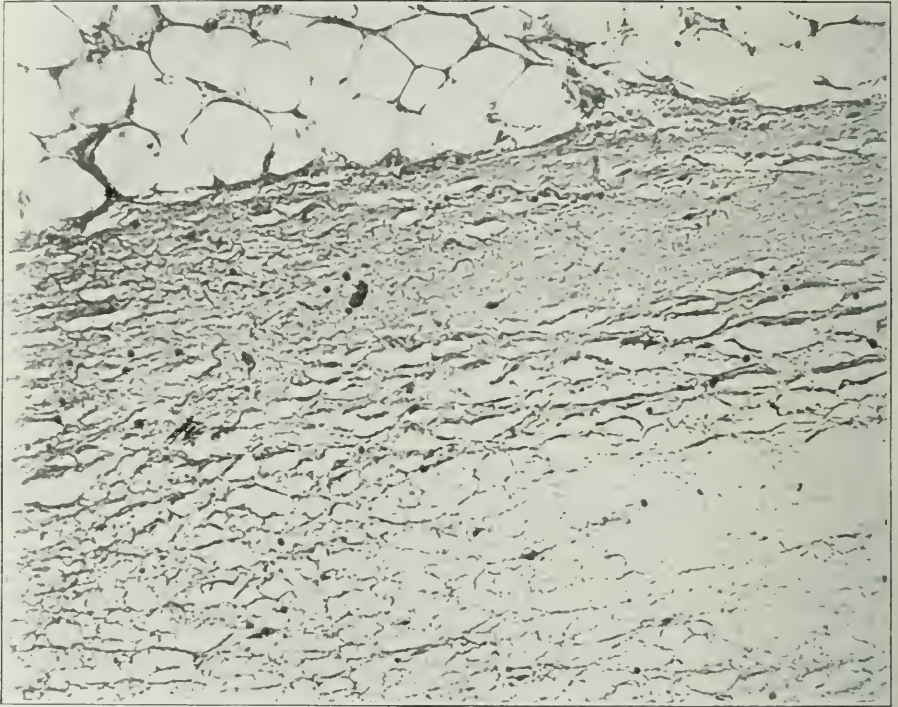


Fig. 29.—Guinea pig five and one-half hours after application of mustard gas to skin of abdomen. Deep subcutaneous edema.

and there is but little leucocyte infiltration. Surrounding this is a narrow zone of less marked necrosis and degeneration. This gradually becomes hyperemic and small hemorrhages by diapedesis may take place from the damaged vessels. In this area there is a more marked infiltration of leucocytes but this rarely becomes diffuse, the leucocytes remaining collected in the neighborhood of the vessels. Outside of this zone the tissues are hyperemic, somewhat edematous and show an increased number of wandering cells for some distance.

3. *Stage of Eschar Formation.*—There gradually begins a separation of the completely necrotic tissue from the living. This eschar consists of the dead epidermis and upper part of the corium, sometimes as far down as the lower borders of the hair follicles. This dries, contracts, becomes leathery, but is held in position by the hairs. There now develops in the neighboring living tissue a productive inflammation. The shrinking of the necrosed tissue and the demarcation



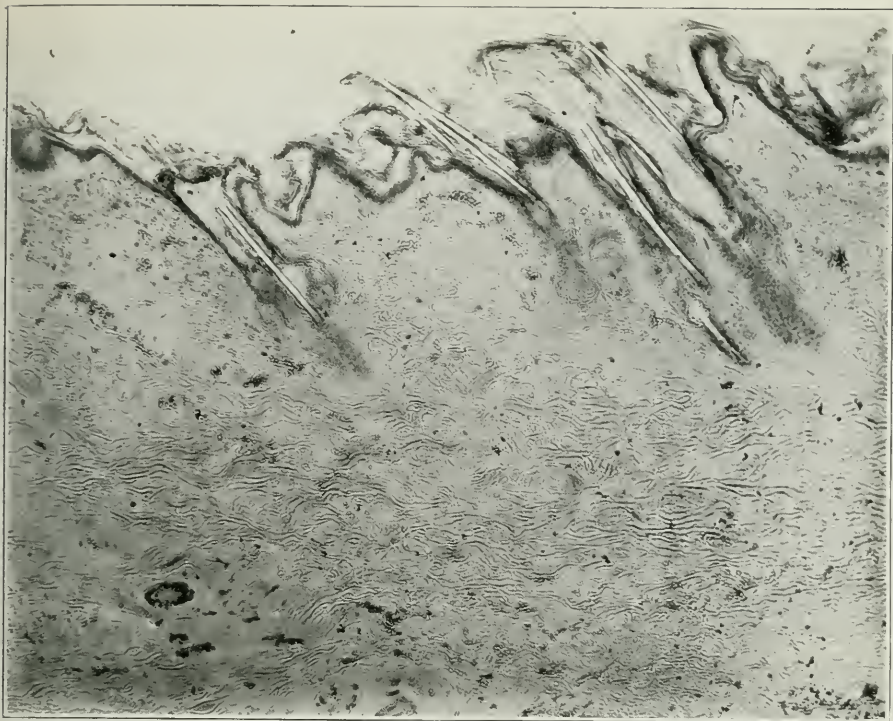


Fig. 30.—Rabbit. Six days after application. Treatment with zinc oxide paste five minutes after use of mustard gas. Center of lesion. Complete necrosis of epidermis, hair follicles and upper portion of corium, extending even to the sweat glands. No reaction.

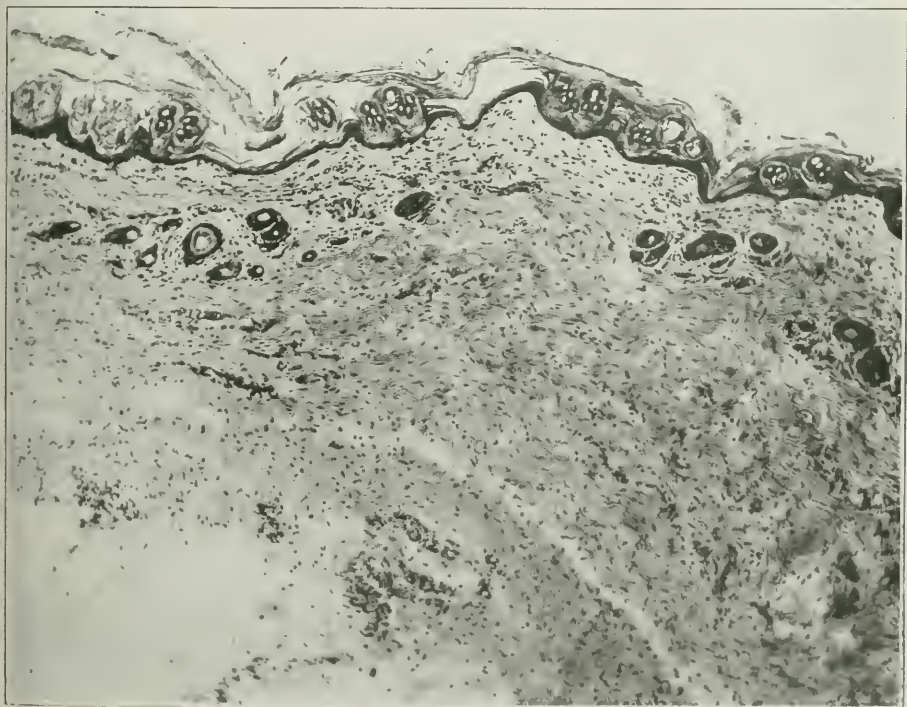


Fig. 31.—Rabbit. Six days after application. Treatment with zinc oxide ointments five minutes after application of mustard gas. There was no edema stage. Epidermis is dead and there is a moderate inflammatory reaction in the corium. Reaction much less intense than in control.

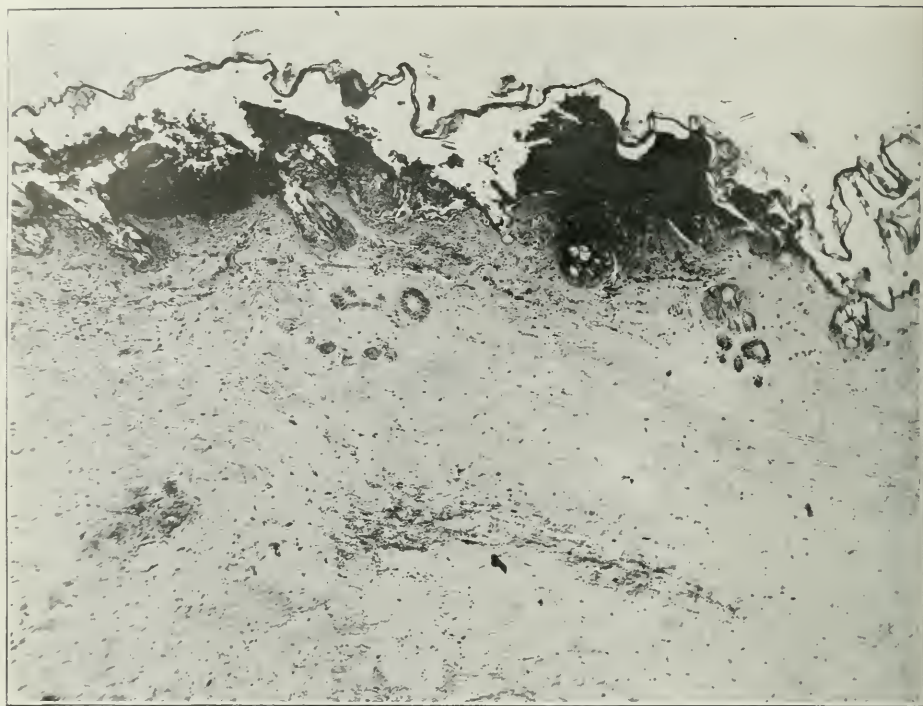


Fig. 32.—Rabbit. Six days after application. Treatment after five minutes with two per cent solution of silver nitrate and five per cent lead acetate. Primary edema was nearly completely controlled but necrosis six days after is marked, extending deep into the corium, with more rapid separation of the slough.



Fig. 33.—Rabbit. Periphery of same lesion as Fig. 32. Area of less damage.



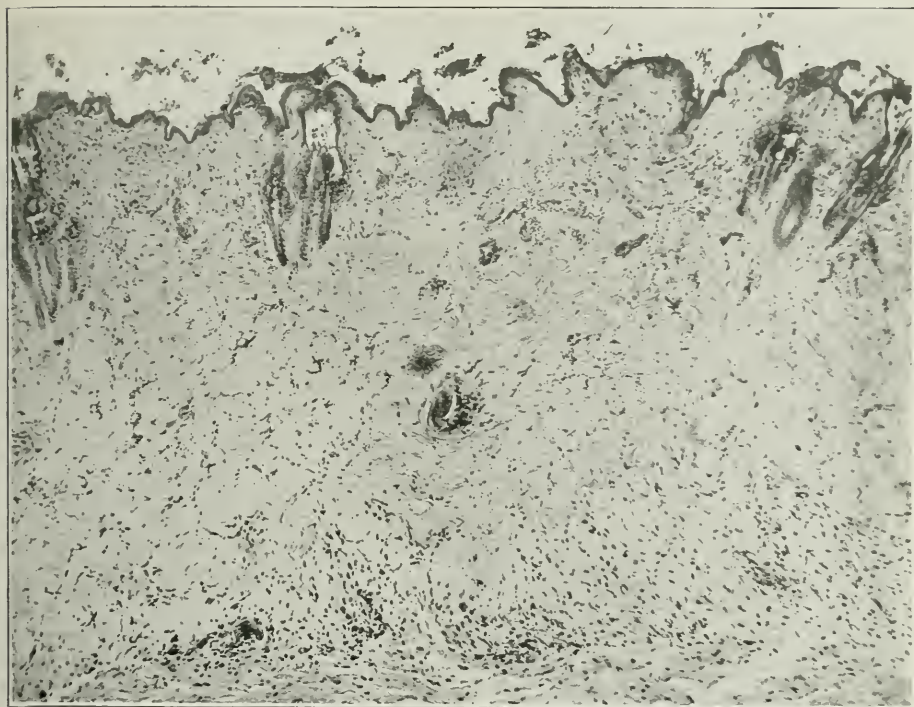


Fig. 34.—Rabbit. Six days after application, untreated. Border of lesion. Necrosis less marked. Beginning repair.



Fig. 35.—Rabbit. Six days after application, untreated. Intermediate zone. Separation of necrotic epidermis and papillary layer with infiltration of leucocytes into the necrotic tissue. Fibroblastic proliferation in lower part of dermis with regeneration of hair follicles. Intense congestion of subcutaneous vessels.

with the surrounding reparative inflammation, progress very slowly until there is a regeneration of the epithelium beneath the eschar. The latter remains adherent, usually until complete repair has taken place. The repair of the epidermis takes place chiefly from the cells remaining in the hair follicles.

(For the histologic changes in animal tissues see Figs. 25 to 36 inclusive.)

#### SUMMARY

1. Dichlorethylsulphide (mustard gas) is an escharotic, specific in its action upon the epidermis and tissues of corium, particularly upon the endothelium of the vessels.

2. The lesion is a chemical burn unlike that produced by heat, electricity,

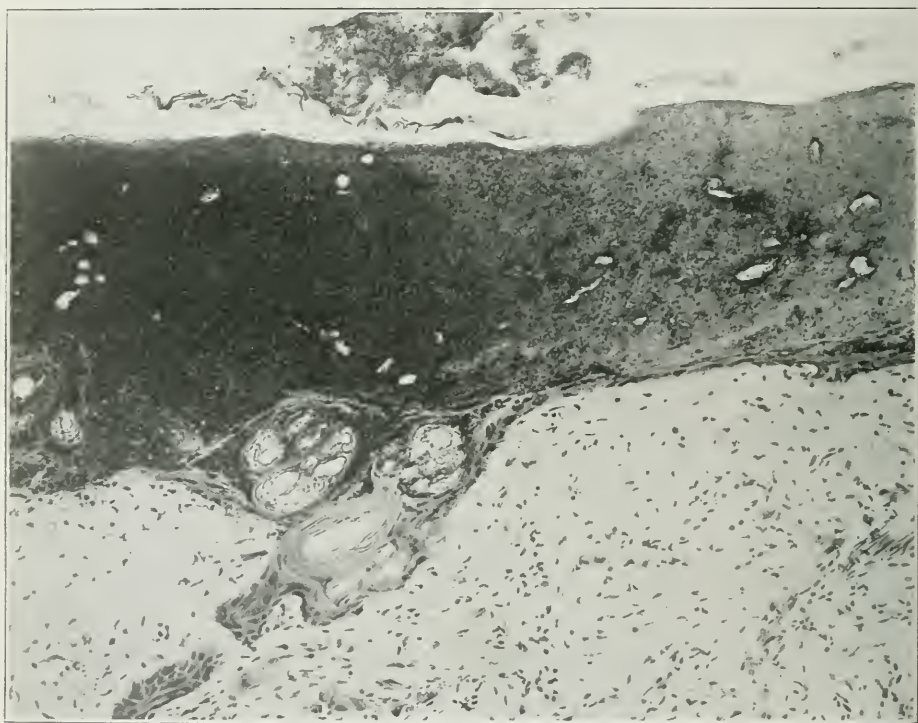


Fig. 36.—Rabbit. Six days after application, untreated. Adherent slough, representing the necrotic epidermis and upper portion of corium, involving the hair follicles.

or the ordinary corrosives such as sulphuric, nitric, and hydrochloric acids or strong alkalis. Of all these agents, the effects are most closely allied to those of hydrochloric acid, but are much greater in intensity. It differs from a heat burn in the absence of thrombosis, in the greater degree of fluid exudation, in the greater moistness of the affected area and in the fact that the necrosis as shown by the loss of nuclei requires hours, or even days, for its complete development. The coagulated, shrunken and cooked appearance of the tissues in heat burns is not apparent in the tissues of mustard gas burns.

3. The vessels in the affected area are severely damaged and collapsed and there is a local anemia in the earlier stages, with a marked fluid exudation and leucocyte migration. The process is nonhemorrhagic and nonthrombosing.

4. In man the necrosis of the epidermis is usually evident in two hours



through the hydropic change in the epithelium and early vesicle formation. There is no deep edema. It is confined to the epidermis and to the papillary layer in the early stages.

5. In animals the intense and deep edema is most striking and altogether different from that seen in man. Vesicle formation was not noted by us in animals.

6. The deep penetration of the smallest quantities applied to the surface is a most striking feature. There is an undoubted entrance through the hair follicles, sebaceous and sweat glands.

7. The slowly progressive character of the necrosis is a specific characteristic, the height of the necrosis being reached five to ten days after application. This may, in part, be explained by contraction and death of the vessels with resulting anemia in the affected area.

8. The painlessness of the lesion is also a marked characteristic. This may be explained by the edema and degeneration of the nerve endings in the affected portion.

9. In none of our animals was there any conjunctivitis or irritation of the respiratory tract produced by the cutaneous applications. We conclude that there is no evidence of metastasis from the local lesion as claimed by both Meyer and Haldane. We believe that the conjunctival and respiratory lesions are due alone to the direct action of mustard gas and when animals are protected from the vapor no lesions in these organs will result, no matter how severe the skin burn.

10. Contrary to the statements of certain English and French observers, the admixture of water does not increase the escharotic action, but if the oil is immediately washed away, the lesion is greatly reduced in intensity. Washing within two minutes with tincture of green soap may entirely prevent the lesion or result in only a slight hyperemia.

11. We believe that the lesions observed in the axilla, between the fingers and toes, around the genitals and between the thighs of men gassed in action are probably due to the greater moisture of these parts from perspiration and the resulting re-resolution of the gas.

12. The slow healing is probably chiefly due to the vessel injury and the relatively slight leucocytic demarcating infiltration. In this respect the lesion is strikingly like an x-ray burn of the skin.

Our thanks are due to Professors Moses Gomberg and William Hale and the Dow Chemical Company for pure and impure dichlorethylsulphide, and to Dr. George Herrmann for aid in making human applications. Report on the pathology of the respiratory lesions and on the production of the conjunctivitis will be made later.

#### BIBLIOGRAPHY

<sup>1</sup>Meyer, Victor: Ber. d. deutsch. chem. Gesellsch., 1886, xix, No. 3, 3259.

<sup>2</sup>Meyer, Victor: Ber. d. deutsch. chem. Gesellsch., 1887, xx, No. 2, 1729.

<sup>3</sup>Giraud, Albert: Jour. de méd. et de chir. prat., Nov. 25, 1917, lxxxviii, 890-895.

<sup>4</sup>Teulière: Jour. de méd. de Bordeaux, Nov., 1917, No. 12, p. 247.

<sup>5</sup>Mandel and Gibson: Jour. Am. Med. Assn., Dec., 1917, lxi, p. 1970.

<sup>6</sup>Haldane: British War Reports.

<sup>7</sup>Kolls and Gilbert: British War Reports; British Medical Reports.

<sup>8</sup>Raper and Ball: Ibid.

<sup>11</sup>Ibid.

<sup>14</sup>McNee: Ibid.

<sup>9</sup>Marshall and Miller: Ibid.

<sup>12</sup>Mackenzie: Ibid.

<sup>15</sup>McLeod: Ibid.

<sup>10</sup>Kolls and Gilbert: Ibid.

<sup>13</sup>Haldane: Ibid.

<sup>16</sup>Dunn: Ibid.

## A DISCUSSION OF THE LIPOIDS CONCERNED IN GROWTH WITH CLINICAL OBSERVATIONS ON THE ACTION OF TETHELIN\*

By E. L. BARNEY. M.S., M.D., SAN FRANCISCO, CAL.

ONE of the most striking features of modern research on growth is the increasing emphasis which is being placed upon the importance of fats in the dietary, not merely from the standpoint of their calorific value, but also as constituting or being associated with substances essential to growth.<sup>1</sup> The view has recently been advanced that many of these essential substances are to be regarded, not as nutritive materials properly so called, but as catalysors of the growth-process,<sup>2</sup> since substances of a fatty nature or commonly associated with fats exist in quantities too minute to be of any nutritive significance yet they are found decidedly to influence the rate of growth in various tissues.

Three of the lipoids especially concerned in the process of growth are cholesterol, lecithin, and tethelin. Although cholesterol is usually classed with the lipoids on account of its solubilities and its invariable association with fatty substances, it is probably a secondary alcohol. Lecithin and cholesterol have been extensively studied. Both are found in nearly all the tissues of the animal body, neither are found in their identical form in plants, although the isomer of cholesterol and substances closely related to lecithin are widely distributed in plants.

Many theories have been advanced as to the function of lecithin. It was suggested by Loew (1899) and again by Reicher (1911) that by the transformation of fatty substances into lecithin, the higher acids are offered to the cells in a soluble form, the same molecules of glycerophosphoric acid serving repeatedly as vehicles for oxidation of molecules of fatty acids; or, in other words, the physiologic oxidation of fats in general is made possible only through combination in lecithin. A number of investigators have suggested that lecithin is accumulated in anticipation of a rapid cell multiplication as the lecithins are most abundant where growth is the most active, and they are decreased as the seat of growth shifts to some other tissue. This is illustrated by the comparative richness in lecithin of the marrow of young bones and its abundance in gland cells, eggs, and spermatozoa. Much experimenting has also been done on the effects of lecithin and other phosphorus compounds on the function of intracellular ferments and on digestion, but in these phases there has been no definite conclusion. However, it has been demonstrated that lecithin, administered by mouth or subcutaneously, is taken up at once by the tissues for their growth.

Yolk of egg is rich in lecithin, and young animals fed on egg yolk grew more rapidly and with better bone development than the control animals (as demonstrated by Cronheim and Müller).

Cholesterol is found in all animal fats and in nearly all the body tissues and fluids. Like lecithin, it is relatively more abundant in the brain than in the other

\*From the Departments of Surgery and Biochemistry of the University of California Medical School, San Francisco, Cal.

organs. Cholesterol forms from twenty to as much as ninety per cent of gallstones.

The numerous recent estimations of the cholesterol content of the blood in pregnancy in its relation to cholelithiasis; in diabetes showing a constant high content, and in pernicious anemia showing a constant low content, leads us to expect that the study of the lipoids will modify the present prognosis of these diseases.

That cholesterol is of much importance in the body's economy is shown by the fact that cholesterol is a constant constituent of all the cells and, when these cells are broken down in life processes, the cholesterol is not excreted but is utilized again in the formation of new cells. According to Kusumoto, one function of the liver is to break down dead cells, the cholesterol of which passes with the bile into the intestine. The cholesterol is reabsorbed and carried with the chyle via the thoracic duct to the blood and so to the various tissues for recombination into the constitution of new cells; thus cholesterol is seemingly accumulated by lack of elimination. With age, the cholesterol content of the body increases. This fact is strongly suggestive when considered with the increased tendency to carcinoma as age advances.

Experiments with the Flexner-Jobling carcinoma in rats have shown that cholesterol definitely accelerates the growth of this carcinoma.<sup>3</sup> But the multiplex character of the growth of animals is shown by the fact that cholesterol retards the normal growth of young mice although it accelerates the growth of the postadolescent mice. Lecithin in opposition to cholesterol, retards the growth of the Flexner-Jobling carcinoma in rats, but accelerates the normal growth of tadpoles, both in weight and linear dimensions.<sup>4</sup>

For the past thirty years, growth has been considered more or less under the influence of the pituitary body. Marie, in 1886, associated gigantism with an oversecretion of the pituitary body during youth, and acromegaly with an overactive secretion during maturity. Since the time of Marie's clinical observations and the first extirpation of the pituitary in the same year by Victor Horsley, there has been a great amount of experimentation in which the work of Cushing is preeminent; and while some very valuable facts were disclosed concerning the posterior lobe of the pituitary, the function of the anterior lobe remained hazy.

The pituitary body develops at the extremity of the notochord and contains rudiments of its origin from ectoderm, mesoderm, and entoderm. The striking feature in the development of the pituitary body is that it originates at the meeting point of that portion of the ovum from which the nervous centers, the alimentary canal, the mouth, and the base of the skull are developed. The posterior lobe of the pituitary develops as the infundibulum and is nervous in origin, and, while it has a marked influence on blood pressure and the contractions of smooth muscles, it is not essential to life. The anterior lobe arises from the pharynx or upper end of the primitive intestine, is distinctly glandular in type, resembling the parathyroids in structure, and it is essential to life.

Extracts or emulsions of the anterior lobe cause a retardation of growth in young animals,<sup>5</sup> but in postadolescent animals growth is accelerated. Extracts

or emulsions of the anterior lobe also cause an acceleration in the growth and an increase in the malignancy of the Flexner-Jobling carcinoma in rats.<sup>6</sup> After considering the action of the lipoids—lecithin and cholesterol—it was a logical hypothesis that the chemical substance of the anterior lobe which influences growth is a lipid. Following up this idea, Robertson, in 1916, published a series of articles in which he described a lipid which he had isolated from the anterior lobe of the pituitary body. Each pituitary yields about 10 milligrams of this substance. It is soluble in alcohol and ether, and, like lecithin, is a fatty substance in combination with phosphorus and nitrogen. Its phosphorus content is 1.4 per cent and for every atom of phosphorus there are four of nitrogen.<sup>7</sup>

This lipid influences growth in the same way as extracts or emulsions of the anterior lobe of the pituitary; that is, it apparently retards growth when administered to animals before the age of adolescence, and accelerates growth in the postadolescent period.<sup>8</sup> It increases the rate of growth of the Flexner-Jobling carcinoma in rats in the same way as whole anterior lobe tissue.<sup>9</sup> In view of these experimental results, Robertson suggests that this lipid is the active principle of the anterior lobe in its influence on growth. It was termed tethelin from *τεθελὺς* "growing." The effect of tethelin on the repair tissue was studied by experiments on mice wounded by the excision of small pieces of skin." Tethelin was administered hypodermically and it showed a stimulating action on tissue repair.

No work has yet been published on the effect of tethelin as an accelerator of repair in cases of delayed union of fractures, but reasoning from the basis that acromegaly is caused by a hypersecretion of the anterior lobe of the pituitary, it would appear that tethelin would have a very great value in certain cases of delayed union. And may we not anticipate less tedious results in the various forms of skin grafting, also in treating gastric ulcers, and in fact in almost all epithelial lesions tending to chronicity.

Following the suggestion received by the experimental work on wounds in mice, it was decided to use tethelin in the treatment of several types of indolent ulcers, the results of which are given below. With one exception, it was not used in any case where the usual ointments and stimulators of granulation tissue had not been extensively tried without success; nor was it used in ulcers for which there is a well-recognized and efficacious treatment—as in luetic ulcers.

The experiments of C.L.A. Schmidt have shown that tethelin is nontoxic and nonantigenic, hence it is therapeutically without danger to the patient.<sup>11</sup>

To reach the limits of the pharmacopeia and to exhaust the patience of the physician probably nothing can surpass certain types of ulcers, and especially when the cases are ambulatory. With leg ulcers, rest is usually of the utmost importance but many patients are unwilling and many more are unable to secure continued rest in bed for a sufficient time.

As each person has a different physiology, so each ulcer differs from every other; some ulcers refuse to heal under treatment that has sufficed in other cases which appear to be similar. All that is needed in some cases is a soothing dressing and a choice may be made from a long list of ointments—zinc oxide



boric, Lassar's paste, bismuth paste, vaseline, etc. For stimulation there is also a goodly number to choose from—silver nitrate, iodine, naphthalene flakes, balsam of Peru scarlet red. The list of wet dressings is equally long. There is likewise great variation in dressings in which the antiseptic feature is prominent—from a solution of iodine and alcohol to Dakin's solution.

The following cases were selected because to each had been applied a variety of common remedies without success, which fact served somewhat as a control.

All the cases were treated in the out-patient department of the University of California Medical School.

Miss S. O., stenographer, age twenty-seven years. Entered surgical clinic, August 23, 1917.

*Diagnosis.*—Chronic pyogenic granuloma on finger.

History negative except that two years before she had vomited a small quantity of blood after several hours of dancing. No medical diagnosis available of her condition at that time. She recovered in a few days and there has been no recurrence.

On the first of July, 1917, a wart on her finger became infected by a splinter. After the infection subsided, an ulcer resulted which did not heal although under the treatment of a physician. After a duration of seven weeks, patient entered the surgical clinic.

Examination showed an ulcer  $1\frac{1}{2}$  cm. in length on the extensor surface, middle phalanx of middle finger of left hand. The ulcer had a fibrous margin and base of gray, soft granulation tissue. Under nitrous-oxide anesthesia, ulcer was curetted August 23; dry gauze dressing applied, followed in twenty-four hours with zinc oxide ointment dressing daily. August 29 the ulcer was irregular in shape, 0.9 cm. wide in lower portion and 0.6 in upper portion, and 1.6 cm. in length. Granulation beginning to appear but surface of ulcer still concave. Gauze wet with a solution of 100 milligrams of tethelin in 5 c.c. of sterile distilled water.

*Second Treatment.*—August 31, powdered tethelin, 100 mg. used.

*Third Treatment.*—September 1, powdered tethelin 100 mg. Granulations level with skin and thin line of epithelium growing in. Ulcer 1.5 and 0.5 in width and 0.8 in length.

*Fourth Treatment.*—September 3, powdered tethelin, 100 mg. Upper portion of ulcer which was 0.5 in width healed, and lower portion now 0.5 cm. in diameter.

*Fifth Treatment.*—September 5. Ulcer now 0.3 in diameter and healing.

Patient not seen again until September 11 because of disturbances in the city's street car service. But when seen, September 11, the ulcer was covered with a scab formed of powdered tethelin and epithelium. This was removed. The ulcer was entirely healed. Patient was seen one and one-half months later; the ulcer had remained healed and no scar was visible.

E. T., farmer, age twenty-nine years.

*Diagnosis.*—Varicose ulcer.

Entered surgical clinic, August 15, 1917.

*History.*—Twelve years ago had had typhoid fever resulting in a phlebitis in legs. Legs had remained somewhat enlarged since that time. In May, 1917, infected by insect bite followed by scratching, which resulted in an ulcer. Ulcer did not heal. Three months later, Aug. 8, 1917, he entered the University of California Hospital and the ulcer was curetted. Wassermann negative. Transferred to the out-patient department, Aug. 15, 1917. Ulcer dressed in surgical clinic with Lassar's paste. Under this treatment ulcer was healing slowly. After two weeks of this treatment, August 29, when tethelin was first used, the surgical condition was as follows: On inner and anterior surface of left leg, halfway between knee and ankle was an ulcer 1 cm. in diameter, base filled with healthy granulation tissue, but still slightly below the level of the skin. Area about ulcer indurated, veins varicose.

*First Treatment.*—August 29, ulcer 1 cm. in diameter. 100 mg. of tethelin in 5 c.c. sterile distilled water on gauze applied to ulcer.

*Second Treatment.*—August 30, ulcer was covered with epithelium except for small center of 2 mm. The epithelium appeared to lie in several layers and was comparable to a growth of rapidly growing bacteria as staphylococcus. Wet dressing of tethelin 100 mg. again used.

*Third Treatment.*—September 1. ulcer covered with epithelium and surface slightly convex. Powdered tethelin applied.

September 3. Ulcer healed. Patient discharged.

W. A., waiter, age forty-two years.

*Diagnosis.*—Indolent pyogenic ulcer.

Entered surgical clinic Sept. 17, 1917.

*History.*—Patient entered Skin Clinic August 20, 1917. Complaint—ulcer on leg. Three weeks previously, infected leg and ulcer resulted. Upon entering skin clinic ulcer was 4 cm. in diameter. Diagnosed as pyogenic ulcer and the surrounding area as an impetiginous dermatitis. Wassermann taken in June, 1917, at a private laboratory, was negative. The ulcer was treated in the skin clinic with Lassar's paste and ammoniated mercury. Patient was given a prescription of ammoniated mercury and diachylon ointment to be applied twice a day. Under this treatment the ulcer healed rapidly for the first week but after that it healed more slowly, and finally during the last week remained fairly stationary.

On September 17, after four weeks in the clinic, patient was transferred to surgical clinic for treatment with tethelin. The ulcer was at that time but  $1 \times 1\frac{1}{2}$  cm. and seemed to be healing, although it was recorded as being about that size for a week past.

September 17, wet dressing, 100 mg. tethelin applied.

September 18, same treatment. Granulations healthy.

September 19, powdered tethelin. Granulations level with skin and epithelium growing in.

Following the treatment with the powdered tethelin the granulations immediately broke down. The two cases preceding this had been treated with tethelin powder with good results but this particular ulcer was set back by it, probably on account of the irritation due to contamination of the preparation by a small proportion of inorganic salts (sodium sulphate). Tethelin in solution was again used and in 24 hours the granulations were again healthy. The solution was continued for the next week and although the granulations continued of healthy appearance, the epithelium failed to grow.

October 5, 100 mg. tethelin was injected beneath ulcer and a dry gauze dressing used.

October 8, second subcutaneous injection given beneath ulcer which in the past three days had decreased one-half in size.

October 12, third subcutaneous injection given beneath ulcer. Blood for Wassermann taken to complete record.

October 15, ulcer healed. Wassermann one-plus. During the time the patient was in attendance at the clinic, he was working as a waiter, which employment is least suited to the healing of an ulcer.

C. G., schoolgirl, age sixteen years. Entered surgical clinic Oct. 20, 1917.

*Diagnosis.*—Ulcer on leg.

Two months before entering the clinic, she struck her shin on a box. Hematoma formed, became infected and ulcer resulted. Two months of treatment by a physician before entering clinic.

Ulcer at first examination in clinic was irregular in shape, 2 cm. in diameter, deeply sunken below level of skin; unhealthy base covered with sluggish granulations, thickened margin. Ulcer situated on crest of tibia about 12 cm. above ankle.

Subcutaneous injections of tethelin, 100 mg. were given along margin of ulcer until red granulations appeared. Then tethelin used as a wet dressing. Ulcer continued to heal.

October 31, tethelin mixed with lanolin applied to ulcer. Several days later, patient fell on school ground, bruising ulcer. After November 15 tethelin no longer used, ulcer was healed all but 5 mm. Patient given lanolin to use at home.

J. N., Italian girl, age sixteen years. Entered surgical clinic May 29, 1916.

*Diagnosis.*—Ulcer on leg.

*History.*—Ulcer began eleven years ago, 1906, when patient was six years old. It began near the right internal malleolus but spread upward, gradually involving nearly all of the anterior aspect of the leg. Not treated for one year; then operated on by a surgeon in Tennessee, who removed part of the middle third of the tibia. Wound not entirely healed until two years later. Remained healed for nearly seven years, but in November, 1915, after trauma to leg, a small ulcer appeared on the site of the old operative scar. This second ulcer gradually increased in size.

When patient entered clinic, six months after ulcer appeared, there was a large irregular ulcer on inner surface of upper and middle third of right leg, with a punched

out border and slight purulent discharge. Wassermann in blood at this time negative. Ulcer was dressed with ammoniated mercury ointment and patient given potassium iodide and mercuric chloride.

During the next three months, until September 2, 1916, the patient continued mixed treatment and the ulcer received a variety of medications, including balsam of Peru and zinc oxide ointment, aristol, strapping with zinc oxide tape, and ammoniated mercury ointment; the last being used the greater part of the time but the ulcer showed no constant tendency to heal. After this period, Sept. 2, 1916, the same dressings were continued but more vigorous luetic treatment was adopted. Though the Wassermann had been negative and radiograms of the bones of the other leg, the clavicles and the humeri were also negative, yet a radiogram of the right leg showed the tibia to be somewhat enlarged and bowed. It was of course impossible to estimate how much of this bowing and enlargement was due to the operative measures at seven years of age.

The more energetic luetic treatment consisted of  $\frac{1}{2}$  c.c. of mercuric salicylate, of 20 per cent strength, injected once a week into the gluteal muscles. In the record is noted a remarkable improvement in the ulcer after two administrations of the mercuric salicylate, but a month later it was recorded that, although the wound was covered with healthy granulations, the epithelium grew very slowly. Two months after the mercuric salicylate was begun, potassium iodide by mouth was administered up to 45 gtt. daily. Nearly four months after the first treatment with the mercuric salicylate, the patient was discharged from the surgical clinic, the ulcer being healed, Dec. 26, 1916. The potassium iodide was discontinued but the mercuric salicylate was continued. The ulcer remained healed eight weeks until February, 1917, when the skin was again traumatized and a new ulcer formed on the old scar. The administration of mercuric salicylate was continued but the ulcer continued to increase in size. On March 13, 1917, potassium iodide was resumed and gradually increased to 60 gtt. daily. Salvarsan was also added to the treatment. The ulcer continued to show no constant tendency to heal.

From August 16, 1917, the local treatment of the ulcer was augmented by daily exposures to alpine light for ten to twenty minutes and dressed with Lassar's paste (without salicylic acid). The alpine light was continued for one month, during which time the ulcer showed some improvement.

On Sept. 13, 1917, all luetic treatment and the alpine light were stopped, and treatment with tethelin commenced. During the year, from Sept. 2, 1916, to Sept. 13, 1917, the patient had received 34 injections of  $\frac{1}{2}$  c.c. of mercuric salicylate in 20 per cent strength, and, for the greater part of the time, a saturated solution of potassium iodide up to 60 gtt. daily, also four intravenous injections of salvarsan. And during that year of luetic treatment, the ulcer had been well for a period of eight weeks only.

At the time, Sept. 13, 1917, when all luetic treatment was stopped and treatment with tethelin begun, there were two ulcers close to the inner margin of the crest of the tibia in the middle third of the leg. The upper ulcer was of crescent shape, 3.5 cm. in length and its greatest width was 1 cm. The granulations were uneven and the margin ragged and unhealthy. The lower ulcer, lying 1 cm. inferiorly was 2.1 cm. in length and 1.8 cm. in width, was healthy and the epithelium was growing in. The ulcers were extremely sensitive to touch.

For one week, gauze wet with a solution of 200 mg. of tethelin in 10 c.c. of sterile distilled water was daily applied to the wound. For the first four days, there was a marked improvement but by the end of the eighth day the new epithelium was breaking down in one area as fast as it grew in another. Powdered tethelin was then used and this proved too irritating, and the granulations broke down. For a week no tethelin was applied but aristol was dusted over the ulcer. Under this treatment the ulcer increased in size and lost all appearance of healing. At the end of a week of treatment with aristol, Oct. 3, 1917, the two ulcers had united forming an area 7 cm. in length and 2 cm. in width; the granulations were uneven, there was considerable secretion, and the patches of epithelium were defoliating. On Oct. 3, subcutaneous injections of 100 mg. of tethelin were given near the margin of the ulcer. Dry gauze dressing. This treatment continued for one week; ulcer improved but the dry gauze injured the new epithelium. On Oct. 10, therefore, wet dressings of 100 mg. in 10 c.c. of sterile distilled water (twice the dilution before used) were employed in addition to the subcutaneous injections of tethelin. Two weeks later, although the ulcer was healthy and healing, it was thought to lessen trauma to the new tissue by using a mixture of tethelin in some fatty substance. Lanolin was chosen, as the tethelin is remarkably hygroscopic and lanolin capable of dissolving water. This mixture worked remarkably well. On Nov. 17, 1917, the ulcer was but  $\frac{3}{4}$



cm. in length. On Nov. 22, 1917, it was but 1 cm. in diameter, there being a reduction in length from 7 cm. to 1 cm. in six weeks.

On Nov. 30, the patient called at the clinic, after a holiday, with the skin lacerated just below the knee and the area of ulceration bruised. No information could be obtained as to how the leg was injured. However, the injury was of no permanent consequence.

During December, lanolin only was used as the available supply of tethelin had been exhausted. On Dec. 30, the ulcer was 0.7 cm. in length and 0.3 cm. in width, having remained nearly stationary for a period of four weeks. Jan. 2, 1918, a few tubes of tethelin were obtained, and the ulcer given two successive treatments with tethelin. By Jan. 7, 1918, the ulcer had healed and the case was dismissed. Four weeks later, Feb. 2, the patient reported to the clinic. The ulcer had remained healed.

There is no doubt that this ulcer will recur if the crest of the tibia is traumatized. When healed, the epithelium over an area of 27 cm. is closely adherent to the periosteum of the tibia; the blood supply is very deficient, there is no subcutaneous tissue, and unless this portion of the leg is covered by a protective dressing, the ulcer will sooner or later recur.

There has been a certain satisfaction in healing this ulcer with tethelin as it was nearly a year's duration and had run the whole gamut of ulcer treatment.

In a case of Basin's disease in a child six years old, tethelin was also tried. Here there were several ulcers and a control could be had. Tethelin was tried for two weeks without success. Other forms of local treatment were also unavailing until the patient was given bichloride baths.

Mrs. H., age sixty years. Admitted to clinic Oct. 28, 1917.

*Complaint*—Several ulcers in old scar.

History dating to childhood. No Wassermann taken. Solution of tethelin used over a period of two weeks. The inflammation surrounding the ulcers subsided and one small ulcer showed new granulation tissue but after two weeks of treatment the patient did not return to the clinic.

D. E., age eleven years. Treatment begun Aug. 29, 1917.

Tethelin was used in solution on a healthy operative wound on the thigh over an area from which a Wolfe graft had been taken. In two treatments the granulations became exuberant and had to be cauterized with silver nitrate. As there was no control, the results in this case seem only to have shown that tethelin did not cause a retardation of the growth of the granulation tissue.

#### SUMMARY

1. The significance of lipoids in the various processes related to growth is discussed.

2. Evidence is advanced of the efficiency of tethelin, a lipoid extracted from the anterior lobe of the pituitary body, in the treatment of chronic ulcers.

#### BIBLIOGRAPHY

- <sup>1</sup>Hopkins, F. G.: Jour. Physiol., 1912, xlv, 425.
- McCollum, E. V., and Davis, M.: Jour. Biol. Chem., 1913, xv, 167.
- MacArthur, C. G., and Luckett, C. L.: Jour. Biol. Chem., 1915, xx, 161.
- <sup>2</sup>Robertson, T. B.: Jour. Biol. Chem., 1916, xxiv, 347, 363, 385, 397, 409; Ibid., 1916, xxv, 635, 647, 663; Endocrinology, 1917, i, 24.
- <sup>3</sup>Robertson, T. B., and Burnett, Theo. C.: Jour. Exper. Med., 1913, xvii, 344.
- <sup>4</sup>King, H. D.: Biol. Bull., 1907, xiii, 40.
- Johnson, M. S.: Univ. of California Publ. Zoology, 1913, xi, 53.
- <sup>5</sup>Aldrich, T. B.: Amer. Jour. Physiol., 1912, xxxi, 94.
- Schafer, E. A.: Quart. Jour. Exper. Physiol., 1912, v, 203.
- Wulzen, R.: Amer. Jour. Physiol., 1914, xxxiv, 127.
- Robertson, T. B.: Jour. Biol. Chem., 1916, xxiv, 385.
- <sup>6</sup>Robertson, T. B., and Burnett, Theo. C.: Jour. Exper. Med., 1915, xxi, 280.
- <sup>7</sup>Robertson, T. B.: Jour. Biol. Chem., 1916, xxiv, 409.
- <sup>8</sup>Robertson, T. B.: Jour. Biol. Chem., 1916, xxiv, 397.
- <sup>9</sup>Robertson, T. B., and Burnett, Theo. C.: Jour. Exper. Med., 1916, xxiii, 631.
- <sup>10</sup>Robertson, T. B.: Jour. Amer. Med. Assn., 1916, lxxv, 1009.
- <sup>11</sup>Schmidt, C. L. A.: Jour. Lab. and Clin. Med., 1917, ii, 711.



# THE GONOCOCCIDAL ACTION OF PROTEIN SILVER SOLUTION IN VITRO\*

WITH SPECIAL REFERENCE TO THE SILVER-FASTNESS OF GONOCOCCI

By HARRY CULVER, M.D., CHICAGO, ILL.

THE ideal therapeutic agent for a local infection is one which has an elective action against the microorganisms without doing damage to the tissues. Numerous chemical agents when in sufficient concentration *in vitro*, have marked destructive action on many pathogenic bacteria but the concentration contraindicates their use in infected tissues.

The gonococcus has very little resistance to many ordinary chemicals, used as germicides, its tolerance to protein silver salts being so meager that these substances might be known as specifics for gonococcal infection, were it possible for the silver solution in proper concentration to come in contact with all gonococci in the tissues. However, it has been repeatedly demonstrated, both experimentally and clinically, that after thirty-six to forty-eight hours after the inoculation of the mucous membrane with gonococci the organisms have penetrated between the epithelial cells and lie safely protected in the submucosal connective tissues. Chemicals which would reach, and destroy gonococci in the urethra, or even those between the epithelial cells, may not have the penetrating power necessary to reach the submucosa, or may reach it so diluted by tissue juices, that the concentration is not sufficient to kill the organisms with which they come in contact.

Scholtz\* has found that by injecting the urethra of a man suffering from acute gonorrhea, with protargol 1%-3% argentamine or silver nitrate 1 to 3000, and holding it for 20 to 30 minutes, after which the medicament was removed by urethral irrigation, the surface epithelium, removed with a curette, contained many intra- and extracellular gonococci which could not be artificially cultivated. He therefore concluded that the organisms of the surface mucous membrane were dead. He also found by similar methods that the deeper mucosa and submucosal tissues contained living, cultivatable organisms.

The number of silver preparations now being used is large. From their chemical structure and reported therapeutic worth, it must be concluded that they all have more or less value as gonococcidal agents. One is frequently confronted with the question of relative gonococcidal strength of these various silver preparations. Certain strengths of each of these salts are recommended by manufacturers for clinical use, but no publications are available of the relative activity of these preparations *in vitro*, therefore four commonly used silver salts argyrol, silvol, protargol, and nargol were taken as being a fairly representative list from this group for standardization.

\*From the Department of Pathology and Bacteriology, University of Illinois, College of Medicine, Chicago, Ill.

\*Prinzipien der Gon-Be-handlung, Deutsch. med. Wchnschr., 1905, No. 23, p. 935.

## TECHNIC

Ten small test tubes are placed in a rack and in each is put a standard amount of sterile hydrocele broth. A determined amount of freshly prepared silver solution is added to each tube to make the silver in the following dilutions 2%-1%- $\frac{1}{2}$ %- $\frac{1}{4}$ %, etc., to 1/128% with one tube of hydrocele broth as a control. To each of these tubes is added a standard amount of a suspension of a 24-hour growth of gonococci. As the organisms are added the tube is agitated to get an even mixture and at once a drop of this mixture is inoculated on a long blood agar slant. The slant is covered with either the water of condensation, already in the tube, or a few drops of sterile water to dilute the gonococcal agent brought over with the organisms. Similar tube inoculations are made after the various periods of exposure of the gonococci to the gonococcal agent, which periods were 5 min., 10 min., 30 min., and one hour. All inoculated tubes were incubated at 37° C. for 48 hours and the colonies on the tubes were counted or approximated. To be relatively certain of the accuracy of the results, these experiments were repeated six times for each silver salt represented, and Table I represents average experiments selected from the above mentioned list.

TABLE I

SHOWING THE RELATIVE ACTIVITY OF ARGYROL, PROTARGOL, SILVOL AND NARGOL AGAINST GONOCOCCI IN VITRO

ARGYROL	AT	5	10	30	1
	ONCE	MIN.	MIN.	MIN.	HR.
2%	0	0	0	0	0
1%	0	0	0	0	0
$\frac{1}{2}$ %	4	0	0	0	0
$\frac{1}{4}$ %	11	10	0	0	0
$\frac{1}{8}$ %	10	12	25	0	0
1/16%	25	10	20	25	0
1/32%	200	400	10	25	10
1/64%	200	50	15	500	200
1/128%	1000	100	400	100	200
Control	2000	1000	1000	1000	1500

SILVOL	AT	5	10	30	1
	ONCE	MIN.	MIN.	MIN.	HR.
2%	0	0	0	0	0
1%	0	0	0	0	0
$\frac{1}{2}$ %	0	0	0	0	0
$\frac{1}{4}$ %	100	100	0	0	0
$\frac{1}{8}$ %	50	10	15	0	0
1/16%	100	10	25	0	0
1/32%	500	200	250	0	0
1/64%	200	2000	2000	100	0
1/128%	3000	2000	3000	3000	0

PROTARGOL	AT	5	10	30	1
	ONCE	MIN.	MIN.	MIN.	HR.
2%	0	0	0	0	0
1%	0	0	0	0	0
$\frac{1}{2}$ %	0	0	0	0	0
$\frac{1}{4}$ %	0	0	0	0	0
$\frac{1}{8}$ %	50	25	0	0	0
1/16%	50	10	15	0	0
1/32%	500	50	200	0	0
1/64%	300	250	100	100	0
1/128%	1000	200	1000	50	100

NARGOL	AT	5	10	30	1
	ONCE	MIN.	MIN.	MIN.	HR.
2%	0	0	0	0	0
1%	0	0	0	0	0
$\frac{1}{2}$ %	0	0	0	0	0
$\frac{1}{4}$ %	0	0	0	0	0
$\frac{1}{8}$ %	50	100	0	0	0
1/16%	1000	40	0	0	0
1/32%	1000	500	11	0	0
1/64%	2000	50	400	11	0
1/128%	2000	1000	1000	500	100

From this table it can readily be seen that beyond a certain concentration in all of these silver salts an instantaneous exposure is sufficient to kill all gonococci with which they come in contact. This concentration is also seen to be well below that usually used of the same silver solution clinically. How-

ever the exposure of unprotected organisms to the action of germicides *in vitro* is very different from the application of a similar strength of germicide *in vivo* where the organisms may be inaccessible and protected by diluting tissue juices. Nevertheless, the relative action of these silver solutions *in vitro* represents their probable relative action *in vivo*, since their penetrating qualities are not known to differ.

From this table, one must conclude that protargol and nargol have practically the same gonococcidal power, since they both kill in  $\frac{1}{4}$  per cent concentration both on exposure for an instant and also for a five-minute exposure. There is further equality of action demonstrated by longer exposures. Likewise argyrol and silvol are seen to be almost equal in gonococcidal power, the slight advantage being in favor of silvol, since it kills at  $\frac{1}{2}$  per cent both on instantaneous exposure and five minutes' exposure; while argyrol does not completely sterilize instantaneously in  $\frac{1}{2}$  per cent concentration, but does so in five minutes. From a careful analysis of this table then, it is seen that protargol and nargol, equal in gonococcidal activity, are about twice as active as argyrol and silvol.

## EFFECT OF EXPOSURE TO LIGHT AND AGE OF SOLUTION

The above silver salts were prepared in 2 per cent solutions exposed at room temperature to ordinary daylight for two months. The gonococcidal activity of these solutions was tested at various intervals during the two months.

Table II represents the action of these various silver salts at the end of two months.

TABLE II

SHOWING THE ACTION OF OLD SILVER SOLUTIONS ON GONOCOCCI  
(Compare with Table I.)

ARGYROL	AT ONCE	5 MIN.	10 MIN.	30 MIN.	1 HR.
2%	15	0	0	0	0
1%	50	40	0	0	0
$\frac{1}{2}$ %	50	20	10	0	0
$\frac{1}{4}$ %	25	20	50	0	0
$\frac{1}{8}$ %	200	25	20	10	10
1/16%	500	200	200	200	50
1/32%	200	500	200	500	25
1/64%	500	1000	500	500	25
1/128%	1000	1000	1000	1000	1000
Control	2000	2000	2000	2000	1000

SILVOL	AT ONCE	5 MIN.	10 MIN.	30 MIN.	1 HR.
2%	20	0	0	0	0
1%	25	0	0	0	0
$\frac{1}{2}$ %	25	20	0	0	0
$\frac{1}{4}$ %	25	200	50	10	0
$\frac{1}{8}$ %	200	50	10	10	0
1/16%	100	100	50	50	50
1/32%	100	200	50	20	10
1/64%	500	100	100	50	200
1/128%	500	500	500	500	500

PROTAR- GOL	AT ONCE	5 MIN.	10 MIN.	30 MIN.	1 HR.
2%	10	0	0	0	0
1%	15	0	0	0	0
$\frac{1}{2}$ %	40	0	0	0	0
$\frac{1}{4}$ %	50	10	0	0	0
$\frac{1}{8}$ %	1000	100	10	0	0
1/16%	1000	100	10	0	0
1/32%	1000	200	100	10	0
1/64%	2000	1000	500	100	10
1/128%	2000	2000	2000	2000	1000

NARGOL	AT ONCE	5 MIN.	10 MIN.	30 MIN.	1 HR.
2%	20	0	0	0	0
1%	20	0	0	0	0
$\frac{1}{2}$ %	20	0	0	0	0
$\frac{1}{4}$ %	500	200	0	0	0
$\frac{1}{8}$ %	2000	100	0	0	0
1/16%	1000	150	100	0	0
1/32%	1000	500	200	100	0
1/64%	2000	2000	25	100	0
1/128%	2000	2000	2000	2000	1000

From a comparison of Tables I and II it is seen that there is a definite weakening of all the silver solutions tested. This is especially marked in the case of argyrol since its efficiency in a five-minute exposure has decreased 75 per cent, while silvol, protargol, and nargol under the same conditions have each lost one-half of their effectiveness. The loss of strength begins as early as the third day after the solution is made, and from interval experiments similar to that represented in Table II it is found that this decrease in gonococidal activity is almost directly proportional to the age of the solution. From clinical experience it is well recognized that old solutions are more irritating to an inflamed mucosa than freshly prepared ones. Hence the practice of having stock solutions of silver salts is disadvantageous from more than one standpoint. Also the amounts prescribed for home use by the patient should not exceed a quantity enough for a few days use; or better still where possible, the solution might be prepared daily.

## EFFECT OF HEAT

Warm solutions applied to an inflamed mucous membrane are soothing and they also increase the already present hyperemia. These facts together with the fact that gonococci are heat sensitive, make the use of warm silver solutions a logical one. This is in accord with clinical results. The following experiments were made to determine what effect, if any, heat would have on the bactericidal power of the four silver solutions. One per cent solutions were made of nargol, protargol, argyrol, and silvol and at once exposed to a temperature of 120° F. After cooling, the dilutions were made in the usual way and their action on gonococci determined as seen in Table III.

TABLE III

EFFECT OF SILVER SOLUTIONS (AFTER HEATING TO 120° F.) ON GONOCOCCI IN VITRO

ARGYROL	AT ONCE	5 MIN.	10 MIN.	30 MIN.	SILVOL	AT ONCE	5 MIN.	10 MIN.	30 MIN.
2%	0	0	0	0	2%	0	0	0	0
1%	2	0	0	0	1%	10	0	0	0
½%	25	0	0	0	½%	10	0	0	0
¼%	25	10	0	0	¼%	50	25	0	0
⅛%	40	25	2	0	⅛%	100	5	0	0
1/16%	50	500	25	0	1/16%	100	100	25	10
1/32%	500	300	2000	500	1/32%	25	2000	2000	0 (?)
1/64%	2000	5000	200	2000	1/64%	1000	2000	2000	500
1/128%	3000	5000	5000	5000	1/128%	2000	2000	2000	2000
PROTARGOL	AT ONCE	5 MIN.	10 MIN.	30 MIN.	NARGOL	AT ONCE	5 MIN.	10 MIN.	30 MIN.
2%	0	0	0	0	2%	0	0	0	0
1%	0	0	0	0	1%	0	0	0	0
½%	1000	0	0	0	½%	1000	0	0	0
¼%	500	500	0	0	¼%	200	0	0	0
⅛%	2000	100	?	0	⅛%	200	100	0	0
1/16%	300	50	25	4	1/16%	1000	50	25	?
1/32%	2000	1000	10	25	1/32%	0 ?	0 ?	25	10
1/64%	2000	2000	2000	25	1/64%	1000	500	500	?
1/128%	2000	2000	2000	1000	1/128%	1000	2000	1000	2000



A comparison of Tables I and III reveals certain apparent facts. Heat tends to reduce the gonococcidal strength of all of the silver solutions tested. This reduction is demonstrated, however, only in the first column where the exposure was for an instant. For argyrol, silvol, and nargol there is no perceptible decrease in the activity in all other interval exposures, while protargol shows a distinct reduction in killing power for every time interval used in the experiment. However, the gonococcidal power of heat, the increased hyperemia produced by, as well as the increased penetrating power of, hot solutions more than compensate for the slight decrease in germicidal strength. Nevertheless repeated heating of the same solution would undoubtedly render it worthless as a gonococcide; hence it would be well not to keep these solutions once they have been heated. Especially is this true of protargol.

#### CHEMICAL-FASTNESS OF GONOCOCCI

Gonococcal infection is encountered which does not respond to repeated application of the same silver solution or any single germicide used locally for that purpose, while immediate response may be observed if the germicide is changed to another member of the same group or one from a different group. This occurrence would lead one to suspect that the gonococci in the deeper tissues have acquired a resistance for the first medicament, by dint of repeated application to these gonococci in the same or increasing concentration, but not of sufficient concentration to kill, this acquired tolerance being applicable only to the particular medicament used and not to other members of the same group, or different groups. Seinsi and Noguchi\* have found that the tolerance of the *Treponema pallida* can be raised to salvarsan to five and one-half times their original tolerance by growing these organisms in gradually increasing strengths of arsenic preparations. In a similar manner they found their tolerance for mercury was raised thirty-five to seventy times. Danyz,† working with anthrax bacilli and arsenic preparations likewise found an acquired tolerance for these organisms against such preparations. In similar experiments gonococci were grown in hydrocele-broth tubes to which had been added various percentages of silver solutions used in the above experiments. These organisms were left in contact with the silver solution media from 30 minutes to one hour, and then plated on blood-agar tubes. The growth from the blood-agar tube, which corresponded to the highest silver solution was used in a similar experiment. By thus transferring gonococci from a silver solution media to a similar media of greater silver concentration, organisms were grown which had a marked increased resistance for this particular silver solution. This silver tolerance is gradually acquired. In the experiments here reported the adaptation took place in the course of two weeks and usually from five to six successive transfers to produce the results shown in Tables IV and V.

\*The Drug Fastness of Spirochetes to Arsenic, Mercurial and Iodide Compounds in Vitro, Jour. Exper. Med., 1917, xxv, 349.

†Immunisation de la Bactériémie charbonneuse contre l'Action du Serum du Rat, Ann. de l'Inst. Pasteur, 1900, xiv, 641.

TABLE IV

REPRESENTS THE ACTION OF ARGYROL, SILVOL AND NARGOL ON STRAINS OF GONOCOCCI IMMUNIZED AGAINST THESE SILVER SOLUTIONS

ARGYROL	AT ONCE	5 MIN.	30 MIN.	SILVOL	AT ONCE	5 MIN.	30 MIN.	1 HR.	NARGOL	AT ONCE	5 MIN.	30 MIN.
1/10%	4000	2000	1000	1/20%	200	200	10	0	1/20%	3000	1000	1000
1/5%	2000	2000	0	1/10%	100	25	20	0	1/10%	3000	2000	2000
1/2%	2000	500	0	1/2%	100	200	10	0	1/5%	50	20	400

TABLE V

REPRESENTS THE ACTION OF NARGOL AND PROTARGOL AGAINST A NARGOL-TOLERANT GONOCOCCUS

NARGOL	AT ONCE	5 MIN.	30 MIN.	PROTARGOL	AT ONCE	5 MIN.	30 MIN.
1/20%	3000	1000	1000	1/20%	1000	100	10
1/10%	3000	2000	2000	1/10%	50	10	0
1/5%	50	20	400	1/5%	0	0	0

The increased resistance acquired by the gonococci is not great in any instance; but no doubt this resistance could be further increased by continuing the experiments. However, Table IV compared with Table I gives sufficient evidence that such an adaptation does take place since the silver solutions are only one-half as effective against the treated as against the untreated stain.

The possibility of a general tolerance to all silver solutions must be considered once an organism gains resistance to any one silver solution. In order to investigate this aspect of the question a nargol-tolerant organism (10 per cent in 30 minutes exposure) was tested against various concentrations of nargol and protargol as represented in Table V.

While this particular culture of gonococci exhibits a considerable resistance against the action of nargol; there is no such resistance produced against the action of protargol which acts on this strain of gonococci as it does on an ordinary strain. Similar experiments for organisms treated with other silvers have been made and tested as in Table V, against the same and different silver solutions, with no variance from the general conclusions that may be drawn from this table.

#### SUMMARY

From repeated experiments *in vitro*, it is found that protargol and nargol are practically equal in effectiveness as gonococcidal agents, while argyrol and silvol are likewise of almost the same strength. The first two being about two times as strong as the last two salts.

A considerable deterioration takes place in each of these silver solution after exposure to light and age. This is especially marked with argyrol which loses 75 per cent of its gonococcidal strength with such an exposure for two months; while nargol, protargol, and silvol loses 50 per cent of strength under similar conditions.

Heating the solutions of the above-mentioned silver salts reduces their action as chemical gonococcides, which reduction is most marked in protargol. The therapeutic usefulness of physical heat in gonococcus infection, however,

probably more than recompenses the slight gonococidal reduction of the solutions.

Continuous or intermittent exposure of a culture of gonococci to gradually increasing concentration of any single silver solution *in vitro* produces very apparent increased tolerance in the organisms for the specific silver solution used. This increased tolerance is not general for all protein silver solutions; as any other member of this silver group will act against this drug-fast organism as against any untreated organism. These experimental facts are of considerable clinical value since it is at once apparent that old silver solutions should not be used, also that heating to 120° F. does not reduce the chemical effectiveness of these solutions, however a silver solution once heated could not be repeatedly heated without reducing its therapeutic worth. Gonococcal infection which is very resistant to one particular gonococcide, may be so, due to an increasing tolerance of the infecting gonococci for the particular gonococcide used. The proper clinical result may be obtained by changing the gonococcide to one of the same group or different group.

---

## THE DEMAND FOR AND TRAINING OF LABORATORY TECHNICIANS\*

---

By JOHN A. KOLMER, M.D., PHILADELPHIA, PA.

---

FOR many years persons who were not graduates in medicine, and especially young women, have been trained in certain laboratory work as in histological methods, particularly in laboratories connected with medical schools and large hospitals and for various other kinds of work in municipal and commercial laboratories. As a general rule the young men and women who have chosen this occupation have proved quite successful and particularly the latter, although opportunities have been quite limited and remuneration comparatively low.

The demand for properly trained laboratory aids or technicians has been readily increasing in the last few years due, not only to the organization of more municipal laboratories, but more particularly to the establishment of laboratories in hospitals and other institutions for the care of the sick and injured and of private laboratories for individual physicians and surgeons. In Pennsylvania the demand has been particularly heavy because about two years ago the legislature enacted a law requiring all hospitals and institutions, particularly those receiving state aid, to install and equip an adequate laboratory and to employ a laboratory technician on a full-time basis. An inspection of all the institutions of the state by the Board of Medical Licensure, under the presidency of Dr. J. M. Baldy, has shown that the hospitals located in the cities, towns and rural districts, with the exception of relatively few of the larger institutions located in the principal cities, were without adequate laboratory facilities for the study and diagnosis of persons seeking advice and treatment, or for an adequate laboratory service and

---

\*From the Department of Pathology and Bacteriology in the Philadelphia Polyclinic.

training for their internes. All institutions caring for the sick and injured, and particularly those offering internships, were classified and required by the Board to install a laboratory adequately equipped and conducted by a pathologist and technician; furthermore, the staffs of the respective institutions were urged and requested to avail themselves of these laboratory facilities.

The law of Pennsylvania requires that the recently graduated physician seeking a license to practice medicine and surgery must have graduated from an acceptable school and have served at least one year's internship in a hospital, and that this internship must have been spent in a hospital acceptable to the Board; one of the requirements exacted by the Board is that the hospital have a properly conducted laboratory offering a satisfactory laboratory service to the interne. Hospitals failing to install and conduct a laboratory meeting the requirements of the Board of Medical Licensure are refused recognition and their internes are regarded as ineligible for examination for a license to practice medicine and surgery.

This law of Pennsylvania, which marks an epoch in medical education, naturally worked a hardship on many hospitals and particularly the smaller and poorer institutions, but an analysis of the situation shows very clearly the wisdom in demanding a higher standard of education and experience on the part of the physician seeking to practice medicine and surgery among its citizens. In the present day of refined and practical laboratory methods for the diagnosis of disease not a small part of the education of the medical man is embraced in a working knowledge of laboratory methods in the diagnosis and treatment of disease. In our opinion great credit is due Dr. J. M. Baldy, and other members of the Board of Medical Licensure, for the very important work they have done in raising the standards of hospitals and requirements for license in the State of Pennsylvania, the influence of which will be exerted throughout the United States.

*Furthermore, since the entry of the United States into the Great War, calling many of our pathologists and bacteriologists into the medical department of the Army and Navy, the demand for laboratory technicians has still further increased; if the war continues this demand for technicians will continue to increase not only for the laboratories of institutions, municipalities and private physicians, but probably also for the laboratories of the various hospitals in the Army and Navy and possibly even for the cantonments in this country and the hospitals abroad.*

As a general rule technicians who have been trained in but one phase of laboratory work as in histologic methods, are required only in the larger institutions handling sufficient of this work to occupy the technician's full time; smaller institutions and for private laboratories a broader training and experience is demanded.

The object of this communication is to emphasize the importance of large laboratories, and particularly those connected with teaching hospitals, to undertake the systematic training of properly prepared young men and women and particularly the latter, in laboratory methods in order to meet this increasing demand for laboratory technicians. During the past two years the Polyclinic



has offered courses of instruction which have proved highly successful; more than seventy-five young women have been prepared and are giving efficient and satisfactory services in the laboratories of hospitals and other institutions throughout the United States.

These courses of instruction are intensive and eminently practical with most attention devoted to technic and practical instruction; lectures are reduced to a minimum in order to devote most of the time to practical work. Instruction is given on five or six days in the week and for four to six hours per day. The classes are limited to a few students in order to permit individual instruction and supervision and criticism of practical work; it has not been found practical or advisable to attempt the teaching of large classes. Each course of instruction is designed to give a broad training in order to enable the technician to handle a varied amount of work as is usually encountered in a well conducted and busy laboratory.

Candidates for instruction must be high school graduates or possess the equivalent in preliminary education; a knowledge of chemistry and biology has been found helpful but not essential. Our most successful students have been those who have entered into this work whole-heartedly and with the purpose of making it their profession; a few have subsequently studied medicine. By reason of their patience, attention to details, cleanly habits and the ability to concentrate most young women in our experience have proved apt pupils and well fitted for work as laboratory technicians. These qualities are frequently lacking in men, whereas they can be developed to a high degree in our students and ensure the conduct of laboratory tests and methods in an orderly, painstaking, accurate and thorough manner. It is the common experience of most pathologists that a well-trained woman technician is capable of doing high-grade work equal to and sometimes superior to that of the physicians themselves and particularly of the interne of average ability and training.

In the Polyclinic laboratories the first course of instruction is in *laboratory technic* which includes systematic training in the preparation of the different kinds of culture media; the different methods of fixing, hardening, embedding, cutting and staining tissues; the preparation of stains; sterilization of glassware and similar work of a fundamental nature. At the completion of the course the student is given a thorough practical and written examination and, if these are passed successfully, the student is eligible for the course in *clinical pathology* which includes instruction in the chemical and microscopical examination of urine, blood, gastric contents, feces and clinical bacteriology, the latter including the Widal reaction, examination of cerebrospinal fluid, sputum, cultures and smears. At the completion of this course a thorough examination is given and it is my custom to advise students to accept positions in order to gain wider experience in the methods and tests which they have been taught, and then to return to the laboratories for instruction in complement-fixation technic with particular reference to the *Wassermann* and *gonococcus complement-fixation tests*. Not infrequently the students elect to continue their instruction until the course in the *Wassermann* reaction has been covered. I fully realize the great importance of thorough instruction and particularly practical experience of the technician in the conduct of the *Wassermann* reaction and my experience dur-

ing the past three years, covering the training of sixty-five persons, has shown me that properly qualified young women are readily trained in the preparation and standardization of the various biologic reagents employed in this important and intricate reaction and to conduct the test in a thoroughly reliable manner. Courses of instruction are also offered to persons having laboratory experience in *bacteriology* and the *preparation of bacterial vaccines* and in *advanced clinical pathology*, the latter course embracing instruction in such subjects as the serologic differentiation of pneumococci; the determination of blood sugar, blood urea and similar chemical methods; the preliminary tests for blood transfusion; the bacteriologic and microscopic examination of milk and water and work of a similar nature. As stated above most attention is devoted to a training in methods and technic, although the students also acquire a fairly good knowledge of the clinical applications and importance of the work in which they are being instructed. When their instruction is finally completed I feel confident of their ability to render valuable and efficient service as technicians, although I constantly advise that it is necessary for them to work under supervision until they have acquired further experience and greater confidence in their work and abilities.

A properly trained and experienced technician is capable of handling a large amount of work of a more or less mechanical nature and thereby conserve the time and attention of the pathologist and physician; up to the present time no attempt has been made to train technicians in pathologic histology because this work demands thorough preliminary instruction in normal histology which is offered only in undergraduate medical schools. Therefore, while technicians are being trained to prepare tissues for microscopical examination and to pass upon the quality of their work by microscopic inspection, they are not being trained to diagnose the tissue changes.

The majority of our technicians have been offered salaries averaging \$900.00 a year with maintenance provided in the hospitals in which they are employed; it would appear that this is a fair wage for at least the first year's work. After this time the technician who proves herself worthy, by reason of extended experience and good work, should expect increased remuneration.

It is to be hoped that other states will follow the example of Pennsylvania in requiring her institutions for the care of the sick and injured to offer adequate laboratory facilities for the modern diagnosis and treatment of disease and for the instruction and experience of internes. If each hospital or institution will offer and give the persons seeking their aid, the benefits of laboratory examinations and demand that the physicians and surgeons on the staff shall be acquainted with the possibilities of a good laboratory service in aiding the diagnosis and treatment of disease, the majority of institutions will need a technician; in my opinion it is entirely right and proper to charge persons financially able to afford a private physician, room, and nurse, for the laboratory examination made in order to meet the expense of maintaining a laboratory and the salary of pathologist and technician. In order to meet the increasing demands for laboratory technicians it is hoped that laboratories with ample facilities and material, will offer adequate and comprehensive courses of instruction and abundance of practical work to small classes of properly qualified persons, in order to supply the demand for broadly trained and experienced technicians.

## LABORATORY METHODS

---

### HOOVER'S DIAGNOSTIC SIGNS ELICITED FROM THE MOVEMENTS OF THE RIBS\*

BY R. G. PEARCE, B.A., M.D., CLEVELAND, OHIO

PALPATION and inspection have not played as important a role in the diagnosis of thoracic disease as the methods merit. It is true that most clinicians observe the character of the respiratory movements, any asymmetry of the thorax, the presence or the absence of Litten's sign, or any abnormality of the cardiac impulse. The observations are quickly made, and auscultation and percussion are employed to elicit further pathologic signs. It may be that the invention of methods of percussion and the introduction of the stethoscope have discouraged the use of the eyes and the fingers as instruments of precision. Be this as it may, it is nevertheless true that the older physiologists and physicians are responsible for most of the current teachings of the respiratory movements. We are accordingly ready to welcome any contribution in this field. Recently Dr. C. F. Hoover<sup>1</sup> has reported some very interesting and important physiologic and diagnostic observations on the movements of the upper and lower ribs and the diaphragm in health and in disease.

He calls attention to the fact that the density of the lungs has occupied the attention of the clinician to the exclusion of the no less important attributes of volume and extensibility, and that a definite idea of these latter characteristics affords valuable aid in the diagnosis of pulmonary and thoracic disease, right and left cardiac enlargement, subphrenic and hepatic changes, and paralysis of the intercostal or diaphragmatic muscles.

In the infraclavicular region the ascent of the ribs during inspiration exhibits an undulatory movement which Hoover asserts is the resultant of two motions: the first brought about by the fact that the ribs increase in length from the first to the fifth, so that in their ascent a lower rib has a tendency to override an upper one; and the second produced by a rotation of the ribs in a sternovertebral axis, which gives rise to the so-called bucket-handle movement. In this case the maximum movement of the rib is in that portion farthest from the axis of rotation. On this latter point he apparently differs with Keith,<sup>2</sup> who points out that with the upper ribs there can be no rotation on the sternovertebral axis on account of the manner of articulation with the vertebræ. If we accept Keith's view, the second movement may be due to the fact that the transverse process on the vertebræ in which the convex ovoid process on the tubercle of the rib articulates, is tilted progressively backward as we descend the series, so that the angle at which the ribs are set in the spine progressively increases,

\*From the Cardiorespiratory Laboratory, Medical Service, The Lakeside Hospital, Cleveland, Ohio.

making the axis on which the upper ribs rotate correspond to their necks. In either case the ascent of a lower rib differs from that of an upper one on account of the length of the rib and its axis of rotation on the vertebræ.

Hoover finds that in disease of the pleura, occlusion of the bronchus, inflammatory process of the lung, or displacement of the lung by an enlarged heart or pericardial sac, the extensibility of the lung is diminished before any increase in density is demonstrable, and that an impairment in the extensibility of the lung modifies the normal undulatory movement of the upper five ribs. For this reason a comparison of the undulatory movements in the two sides affords a very delicate method of determining the relative ventilation in the upper lobes. Any disparity in the undulatory movement on the two sides indicates diminished ventilation volume. The phenomenon is best observed from the right side of the patient. The examiner should use his left hand, placing the tip of the ring finger on the second rib at the midclavicular line, the tip of the third finger on the third rib midway between the midclavicular and the anterior axillary line, and the tip of the index finger on the fourth rib in the anterior axillary line. The patient is then instructed to make a moderately deep and rapid inspiration. The movement of each rib succeeds and exceeds that in the rib just above. A moderate impairment in the ventilation of the upper lobes causes the three ribs to move more in unison and the undulatory movement is diminished or lost, although the movement of the ribs may be considerable during inspiration. Hoover finds the test of very great practical use in the diagnosis of early pulmonary tuberculosis; since in this disease diminished extensibility of the lung can be demonstrated long before evidences of alteration in lung volume or density can be elicited.

Duchenne taught that the contraction of the diaphragm produces a separation of the costal margins, because in its descent it presses upon the abdominal viscera contained in its vault and rolls these forward, pushing out the lower ribs; and this teaching is current today. Hoover shows by experimental and clinical observations that this is not the most important or even a necessary factor in the production of the flaring of the costal margins seen in normal inspiration. He finds that the flaring of the costal borders is very prominent in paralysis of the diaphragm or in any condition in which the abdominal pressure is high and the arch of the diaphragm is correspondingly increased. On the other hand, paralysis of the intercostal muscles or any condition which depresses the arch of the diaphragm will result in the approximation of the costal borders to the median line and a decrease in the subcostal angle. The line of traction of the diaphragm is a straight one joining the central tendon with the edge of the ribs. When the diaphragm forms a well-defined arch, its traction is exerted at a disadvantage, and the external intercostals may have the mastery of the movements of the costal borders. In paralysis of the intercostal muscles, however, the diaphragm acts alone and the costal borders are drawn in. Likewise, when the arch of the diaphragm is depressed, the line of traction and the line of the muscular fibers of the diaphragm are more closely approximated. The diaphragm is then able to use its full force against the intercostal muscles, with the result that the costal border moves toward the median line. Depression of the diaphragm to such an extent that it actual



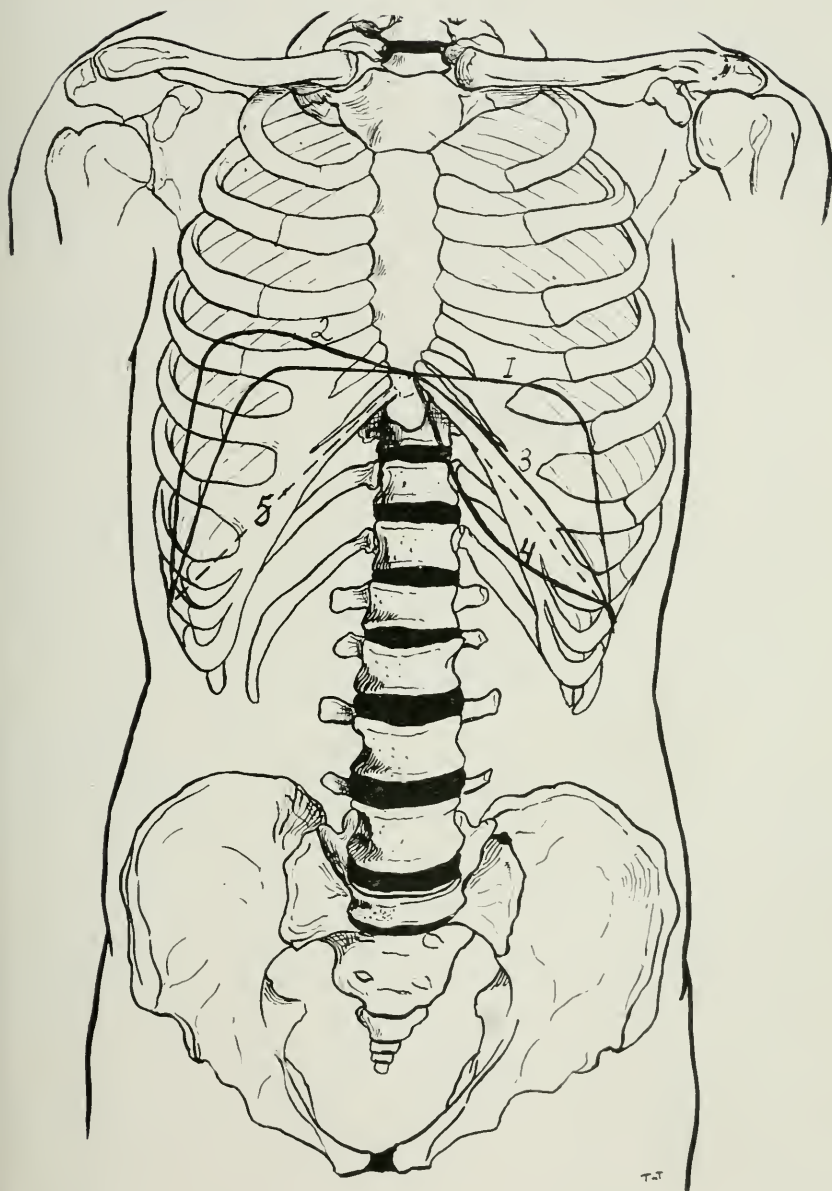


Fig. 1.

1. Normal position of diaphragm. Costal margins move out during inspiration.
2. High position of diaphragm. Normal outward movement of costal margins accentuated.
3. Low position of diaphragm. Costal margins move in during inspiration.
4. Very low position of diaphragm. Costal margins move out during inspiration.
5. Actual line of traction of diaphragm.

arched downwards should result in a flaring of the costal margins during inspiration, since the pull of its muscular fibers would no longer coincide with their line of traction as in the normal position. An example of such a condition was found in a patient with a massive empyema, in whom the movement of the costal margin was normal before the removal of any fluid. Partial removal of the pus resulted in the left costal margin being drawn towards the median line, and further removal brought about a return of the normal flaring. The experimental and clinical observations made by Hoover indicate that the contraction of the diaphragm approximates the costal margins, whereas contraction of the intercostal muscles produces lateral movements. When both act in unison, the resultant movement is dependent on which force is the stronger.

The movements of the margins can be determined by inspection or palpated by placing the thumbs on the costal borders and instructing the patient to take a somewhat deepened inspiration. Fig. 1 illustrates the movement of the costal margins when the diaphragm is low or high due to supra- or infraphrenic diseases, respectively.

The movements of the costal margins interpreted in this light at once serve to give much diagnostic information. Hoover calls special attention to the aid which their movements give in the diagnosis of pleurisy with effusion, empyema, and emphysema. Whenever the lowering of the sheet of the diaphragm is sufficient to give the diaphragm mastery over the intercostal muscles, the subcostal margin moves *in* rather than out during inspiration, and the angle made by them is decreased. Any disparity in the movements of the costal margin on the two sides likewise gives diagnostic data as to unilateral thoracic disease.

Anything which accentuates the arch of the diaphragm will cause the costal margins to flare more than normally. This fact serves to differentiate infra- and supraphrenic diseases such as hepatic enlargement, subphrenic abscess and empyema, pleurisy with effusion, or pericardial effusions. It likewise affords the only infraphrenic signs of diaphragmatic activation.

The curves of the different fibers of the diaphragm vary greatly; the arch is much less marked in that portion of the diaphragm which is attached to the costal margin near the median line than in that which joins the central tendon with the costal border in the axillary line. For this reason the anterior lateral part of the diaphragm requires less depression to give this part of the diaphragm a horizontal position than is required to accomplish the same for parts occupying a more lateral position. The heart overlies this part of the diaphragm and most valuable and direct information can be obtained concerning the size of the pericardial sac and the various characteristics of the heart by careful study of the symmetry of movement of the two costal margins in their upper and median positions.

In any condition giving hypertrophy of the left ventricle—namely, chronic myocarditis, chronic valvular heart disease, or chronic nephritis—the costal margin on the *left* from the ensiform to the ninth rib is restricted in its outward movement during inspiration. In cases of acute dilatation of the right heart auricle, or ventricle, the *right* costal margin from the ensiform to the ninth costal cartilage is restricted. In late valvular disease in which *both* sides a

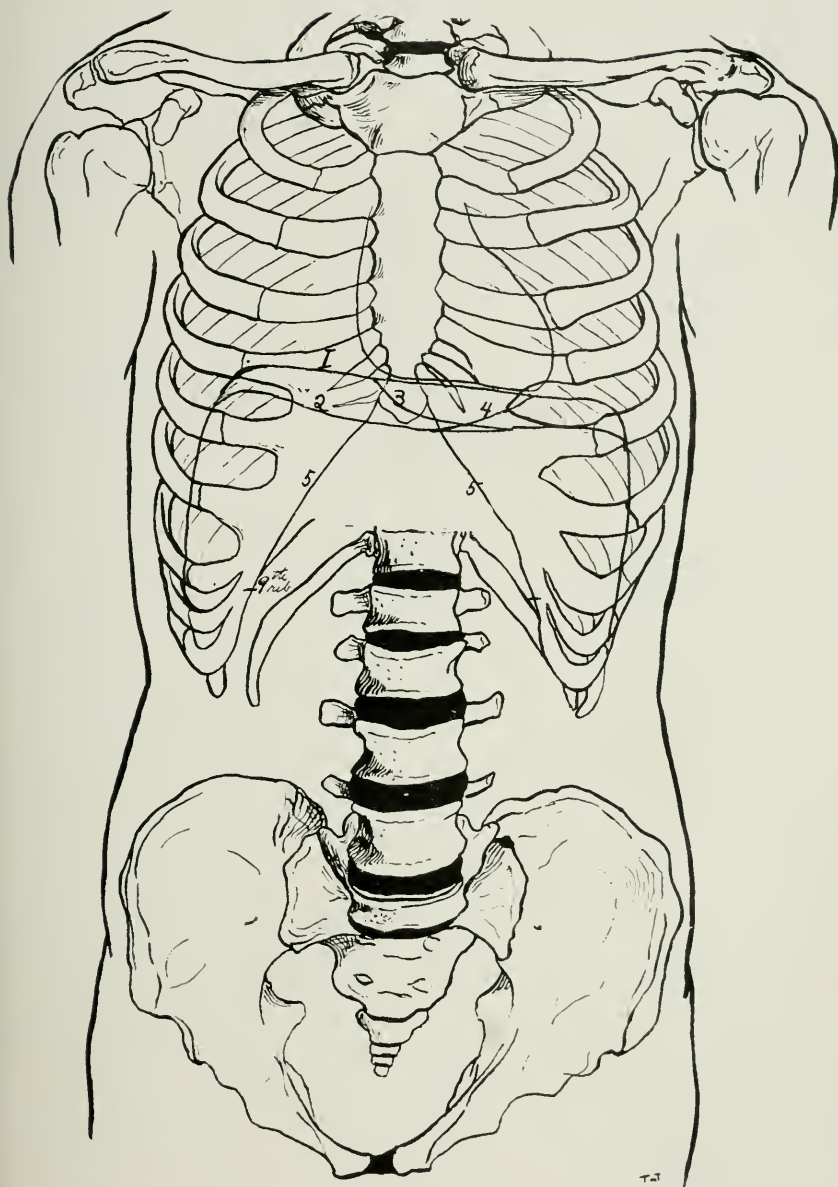


Fig. 2.

1. Normal position of diaphragm. Costal margins move out during inspiration.
2. Position of diaphragm in general cardiac enlargement. Costal margin from ensiform to ninth rib moves toward median line.
3. Position of diaphragm in left-sided cardiac enlargement. Left costal margin is fixed or moves in during inspiration.
4. Position of diaphragm in right-sided cardiac enlargement. Right costal margin is fixed or moves during inspiration.
5. Costal margin.

involved, in general myocarditis, and when there is any increase in the size of the pericardial sac, there is a symmetrical restraint or even approximation of the costal margins during inspiration.

Restriction of the movements of the costal margin on one side must not be taken as absolute indication of cardiac enlargement, but must always be subjected to critical clinical observation. This was well illustrated by two cases which have come under my observation. Both were normal, healthy young men, and in good physical training. In both, the costal margin on the left from the ensiform to the ninth was restricted in its outward movement. Percussion gave the left border outside the nipple line in the sixth interspace. The upper border was found to be under the fourth rib. Auscultatory signs and blood pressure were normal. Fluoroscopic examination confirmed the percussion findings. The explanation of these phenomena lies in the fact that the hearts were occupying a lower and more transverse position than normal, and thus depressed the anterior leaf of the diaphragm. Fig. 2 shows position of the central leaf of the diaphragm in right and left and general cardiac enlargement.

#### BIBLIOGRAPHY

<sup>1</sup>Hoover: Arch. Int. Med., 1917, xx, 701.

<sup>2</sup>Keith: Further Advances in Physiology, Arnold, 1909, p. 182.

#### ERRATUM

In the article "A Clinical Method for Determining the Respiratory Exchange in Man," by R. G. Pearce, B.A., M.D., Cleveland, Ohio, in the April 1918, issue of the JOURNAL, the following table (Table V) was omitted on page 431.

TABLE V

HEAT VALUE OF 1 LITER OF OXYGEN

Cals. for 1 Liter O<sub>2</sub> for Respiratory Quotients from .70 to .97.

0.70		0.75	4.708	0.80	4.770	0.85	4.831	0.90	4.892	0.95	4.953
0.71		0.76	4.720	0.81	4.782	0.86	4.843	0.91	4.904	0.96	4.965
0.72	4.673	0.77	4.733	0.82	4.794	0.87	4.855	0.92	4.917	0.97	4.978
0.73	4.683	0.78	4.746	0.83	4.807	0.88	4.868	0.93	4.930	0	
0.74	4.695	0.79	4.758	0.84	4.819	0.89	4.880	0.94	4.942		



# The Journal of Laboratory and Clinical Medicine

Vol. III.

MAY, 1918

No. 8

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.  
Ann Arbor, Mich.

## ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D.	- - -	ST. LOUIS
HANS ZINSSER, M.D.	- - -	NEW YORK
PAUL G. WOOLLEY, M.D.	- - -	CINCINNATI
FREDERICK P. GAY, M.D.	- - -	BERKELEY, CAL.
J. J. R. MACLEOD, M.B.	- - -	CLEVELAND
ROY G. PEARCE, M.D.	- - -	CLEVELAND
ROGER S. MORRIS, M.D.	- - -	CINCINNATI
GERALD B. WEBB, M.D.	- - -	COLORADO SPRINGS
E. E. SOUTHARD, M.D.	- - -	BOSTON

Contents of this Journal Copyright, 1918, by The C. V. Mosby Company—All Rights Reserved  
Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

## EDITORIALS

### *Investigations on Shock at the Front*

IF intensive concentration on one problem is the most certain way of insuring its solution, the pathogenesis of shock should not for much longer remain a mystery. A great many of the most eminent of American and British physiologists are at present engaged, some of them with experimental shock as observed in their home laboratories, and others with shock as observed at the front. As reports of their findings and experiences come to hand, it is important that they should be carefully studied by those of us who for one reason or another are not directly engaged in the actual observations. We must serve as an audience or tribunal, and thoughtfully study the evidence as it is presented, so that by friendly criticism and suggestion we may take a small though indirect share in the important work.

In a previous number of this JOURNAL, we reviewed a recently published series of papers by Y. Henderson and Haggard in which the claim is reasserted that shock depends upon a condition of so-called acapnia, and in that review it was pointed out that the evidence is insufficient and inapplicable to most forms of

shock. In the present article we would call attention to the much weightier contributions of Prof. W. B. Cannon and his collaborators working on actual cases of shock at the front, and recently reported in the *Journal of the American Medical Association*.<sup>1-6</sup> The investigations referred to are being conducted under the guidance of a special investigating subcommittee of the Medical Research Committee of Great Britain, the personnel of which includes such men as Bayliss, Starling, Sherrington, Cannon and Dale.

In one of these papers<sup>5</sup> Cannon sums up the evidence collected by himself and collaborators, and correlates it with that of previous workers, and it is to this excellent essay that we would particularly refer our readers. The first part concerns itself with a critical review of the existing hypotheses of shock. In criticising the acapnia theory Cannon gives what we believe to be an incorrect interpretation of Henderson's<sup>7</sup> views; to quote, "excessive breathing may greatly lower the  $\text{CO}_2$  of the blood," so that "a protective compensation acidosis is developed to prevent the fatal apnea that might ensue from lack of stimulating H ions." The acidosis is assumed to exist because the  $\text{CO}_2$  content of the plasma is reduced and since Cannon, like most others, has accepted Van Slyke's incomplete definition of acidosis as being a condition in which total plasma carbonates are reduced, it appears that he misinterprets Henderson's new hypothesis. This observer himself claims that during the time that the  $\text{CO}_2$  is being "blown off" there is a relative excess of alkali in the blood and that this excess "passes out of the blood into the tissues." However this may be, Cannon clearly shows the acapnia hypothesis is quite untenable, and he points out that Porter's advocacy of the rebreathing of expired air by patients in shock, so that the  $\text{CO}_2$  content of the blood may be raised, produces no more than temporary relief incident upon the stimulation of the respiratory and vasomotor centers. We shall consider Porter's observations more closely in a future article; suffice it here to state that Cannon does not support the treatment of shock by rebreathing expired air.

The possibility of suprarenal exhaustion is briefly dismissed as obviously untenable, and some space is then devoted to the erstwhile popular hypothesis of nerve cell exhaustion. Besides reviewing the already well-known evidence against this hypothesis, some further facts are cited, among them the observations by Cowell, one of Cannon's collaborators, indicating that the nervous system is functioning in normal fashion, since the intellect is clear, and the patient may retain his muscular vigor to such a degree that two men are necessary to hold him on the stretcher, although no pulse can be felt at the wrist. These facts indicate that there can be no exhaustion at least of the cells of the parts of the nervous system that are higher up than those controlling blood pressure, and if we take this along with the fact, very definitely established by Stewart, Pike and Guthrie some years ago, that the vasomotor center is the last to lose its function during acute cerebral anemia and the first to recover it when blood returns, we see how untenable is the exhaustion hypothesis. Whatever histologic changes may occur in the nerve cells in shock, it is pointed out, they are just as likely to be "the resultant of the low blood pressure as its cause." Persistent low blood pressure, accompanied by acidosis, which Cannon has found to exist, will no doubt ultimately, like hemorrhage itself, lead to secondary cell changes.

The possibility that the low blood pressure in shock is due to cardiac failure is readily dismissed, and attention is then directed to the only factor which remains to explain this low blood pressure; namely, deficiency in the amount of circulating blood. There are in general three prime factors responsible for the blood pressure: peripheral resistance, cardiac action, and amount of circulating fluid. If, as is the case in shock, the first two of these factors are practically unaffected, then we are driven by exclusion to conclude that it must be in the third, the loss of circulating fluid, that the abnormality exists. The conclusion of the earlier workers on the problem (Leonard Hill) must be reverted to, that in shock an animal bleeds into its own blood vessels, and the question that remains to be investigated is the exact location of the vessels. For obvious reasons we may at once dismiss the arteries from consideration. Concerning the veins, more especially those of the capacious splanchnic area, because a condition of experimental shock accompanied by great engorgement of the abdominal veins can be induced in animals by rough handling of the abdominal viscera, it has not uncommonly been assumed that a similar engorgement must also occur in surgical shock. Cannon, quoting interesting observations by Mann on the distribution of the blood after hemorrhage in normal and shocked animals, shows by computation that, if collection of the blood in the veins were really its cause, there would have to be in every case of shock a very evident engorgement of the abdominal veins, which however, as experience at the front testifies, is by no means the case. Neither can the extraabdominal veins serve as the trap for the lost blood, for, if this were so, then tight bandaging of the extremities and abdomen and placing the body in a slanting head-down position should relieve the condition, which however it does not do, although such treatment often gives temporary relief.

The conclusion is arrived at that *the blood stagnation must occur at a part of the vascular system that is beyond the sphere of vasomotor control; that is, in the capillary area.* This is confirmatory of the experimental findings of Mann, that the amount of blood that stagnates in the tissues of a shocked animal may be more than 50 per cent above that of a normal animal. By observations on man it is impossible to secure direct evidence of such capillary stagnation of blood; we must seek to prove it, therefore, by indirect means. But even this is most difficult to find, although the discovery made by Cannon and his coworkers that there is a marked increase in the concentration of the blood during its passage through the capillaries, indicates that something abnormal is occurring there. Briefly stated, it has been found that, whereas in normal persons the corpuscular count, the hemoglobin percentage, and the proportion of corpuscles to plasma (hematocrite readings) are the same in blood taken from capillaries and veins, in shock the values are all greater in capillary blood. This discrepancy between capillary and venous blood is more marked in superficial (say, finger, etc.) than in deeper vascular areas. It indicates that the blood must become concentrated in the capillary areas.

These findings are of an opposite character to those following hemorrhage alone, in which both hemoglobin and corpuscles are reduced. In the cases of hemorrhagic shock observed at the front, the reduction of these values in the

capillary blood was distinctly less than in simple hemorrhage, being masked by the rise induced by the shock, although, as in the cases of uncomplicated shock, the venous blood was much more dilute than the capillary. An important point in the observations is that the venous blood was found to be of normal concentration; that is, the blood going to and coming from the capillary areas, is of the same measurable concentration—so that it must be while it is passing through the capillaries that the blood cells remain behind and accumulate so as to make the blood more concentrated. This does not of course prove that the blood has drained into the capillaries, causing a loss of circulating fluid and a holding back of blood from normal currency. It indicates rather that the holding back must be, not in the capillaries themselves, *but in the tissue cells or spaces.*

These observations are to be considered along with recent work by F. H. Scott,<sup>8</sup> which show that in laboratory animals there is a direct proportion between the arterial blood pressure and the hemoglobin content of the arterial blood, from which it is concluded that the increased pressure forces fluid out of the blood into the tissue spaces. Scott did not, however, compare the capillary and venous bloods in these particular experiments, because he found in other observations, as yet unreported in detail, that the percentage of hemoglobin is practically always identical in capillary and arterial (carotid artery) blood.

As an outcome of this work the question arises as to whether a maintained high arterial pressure in an animal not accustomed to it might cause such a drainage of fluid into the tissues that a concentration of corpuscles in the capillary blood, similar to that observed in shock, might occur, leading to a development of this condition after removal of the exciting cause of the high blood pressure. It is significant in this connection that many of the conditions which are followed by shock in experiments on animals cause a preliminary rise in arterial pressure (e.g., stimulation of afferent nerves.)

As to the causal relationship between low blood pressure and capillary stagnation of corpuscles, Cannon's articles are not quite clear. In one place he states that "besides a low blood pressure \* \* \* there are other conditions that are favorable to capillary stagnation of the corpuscles," and in another, "that the capillary capacity is sufficient to contain the lost blood in shock, and that the chances of its doing so are greater the more concentrated the lost blood becomes." To the present writer it would appear that the fundamental cause for both the lowered pressure and the concentration of blood must be loss of fluid because of its leakage through the capillary walls into the tissues. This leakage is not great enough to produce edema or to make any measurable difference in the percentage of hemoglobin or of corpuscles in the arterial and venous blood of the part (which is not to be wondered at because of the large volume of moving blood at any given moment), but is yet quite sufficient to impede the free movement of blood in the capillaries because of increasing viscosity. Once such a hindering process is set agoing, it will become progressively more pronounced, for the increasing delay in movement will encourage ever larger amounts of fluid to exude; a so-called vicious circle will become established.

That the viscosity of the blood is greatly raised when the corpuscles in



crease in number is a well-known fact, and, as Cannon points out, it is also raised by another of the conditions that are known to predispose to shock; namely, cooling of the surface of the body. Thus, the viscosity of the blood increases 3 per cent with a fall in  $1^{\circ}$  C. of temperature. Shock, as observed at the front, is much more liable to occur in men who have been exposed to extreme surface cooling, and the sweating and absence of shivering which accompanies it often lead to marked lowering of the mouth temperature, readings as low as  $87^{\circ}$  and  $88^{\circ}$  F. having been observed in shocked men. It has moreover been observed by one of Cannon's collaborators (Cowell) that as a wounded man becomes chilled his blood pressure falls, and as he is warmed it may rise again. The greater incidence of shock in cold, wet weather is also significant.

But there is yet another possible factor to be considered; namely, the acidosis which Cannon has shown to exist in shocked men. He found "in general that the lower the blood pressure, the lower the alkaline reserve," as measured by the Van Slyke method.<sup>9</sup> A similar reduction in alkaline reserve was also observed in cases of pure hemorrhage, but, so far as the results go, they tend to show that hemorrhage alone is not attended by as great a loss in the alkaline reserve as in shock (when the blood pressures are equally reduced.) The reduction in  $\text{CO}_2$ -combining power, except in very severe cases, was no greater than might be observed in a normal person after strenuous muscular exercise, but, whereas in the latter case the disturbance is temporary (oxidation quickly removing the organic acids responsible for it, and alkali reserves being called up from the tissues), in shock it steadily goes from bad to worse. Neither was it often observed to be so low as to cause evident stimulation of the respiratory center, although when the  $\text{CO}_2$ -combining power became very low (less than 30 per cent), the average respiratory rate was much greater.

As to the significance of the acidosis, Cannon, taking due cognizance of the work of L. J. Henderson and others, does not draw any hasty conclusions. He points out, for one thing, that the degree of lowering of the alkaline reserve of the circulating blood is probably not sufficient to cause a demonstrable increase in H-ion concentration (because of the buffer action of the blood). There can be little doubt that he is correct in this inference, and that the observations of Crile and others purporting to show an increase in  $\text{C}_{\text{H}}$  are untenable. But, although the blood itself may have no higher a  $\text{C}_{\text{H}}$  than normal, it may be quite otherwise in the tissue fluids, for it is here that the incompletely oxidized acids (lactic, etc.) will be produced. In the stagnant capillary blood, therefore, the  $\text{C}_{\text{H}}$  may be distinctly raised, and as the circulation through these vessels becomes more and more impeded, so will the acidosis of the stagnant blood increase. This local acidosis will be still further encouraged by the lowering of temperature, for it is known that the alkalinity of the body fluids decreases as the temperature falls.

Cannon sums up the possible effects which this local acidosis may bring about. The following are considered: (1) A dilatation of the smaller arterioles and capillaries. Although this local effect of acids on the smaller blood ves-

sels does occur, any increase in  $C_H$  of the blood as a whole would stimulate the vasoconstrictor center and so cause the richly innervated vessels, the arterioles, to become constricted, which they actually are in shock. (2) It will cause the heart to relax more in diastole and discharge less blood in systole. (3) It will increase the viscosity of the blood. Thus, in asphyxia the viscosity of the blood may be double that of normal richly oxygenated blood. This factor, as already explained, will operate to produce stagnation of corpuscles in the capillaries. (4) It will increase the size of the corpuscles (Hamburger), and so help to impede the capillary flow. Some evidence for this effect was secured by observing that "the capillary corpuscular volume was greater compared with the venous corpuscular volume than it should have been according to the rate of corpuscular and venous counts."

To sum up we may quote again: "It seems probable, therefore, that when acidosis is once established it would tend to continue the disturbances of the circulation which have been produced by other conditions."

Attention is directed to the importance of recognizing not only that many factors interact on one another in shock,—vicious circles, they are called,—but also that more careful distinction should be made among the several varieties often loosely classified under this general term. The term "exemia" is suggested as a suitable one to designate the shock due to a holding back of blood from normal currency. It seems to us, however, that, although there are signs that the obscurity surrounding this most mystifying of problems is being gradually dispelled by such researches as have been referred to in this article, we can not as yet see clearly enough the fundamental cause for the condition to justify the coining of a term which after all may refer to but one of its accompanying symptoms.

The researches emphasize the importance of keeping a threatened patient warm and taking means to prevent the development of acidosis. We shall however, defer further reference to therapeutic measures to a future article.

#### BIBLIOGRAPHY

- <sup>1</sup>Fraser, John, and Cowell, E. M., France: Clinical Study of Blood Pressure in Wound Conditions, Jour. Am. Med. Assn., 1918, lxx, 520.
- <sup>2</sup>Cannon, W. B., Frazer, John, and Hooper, A. N., France: Some Alterations in Distribution and Character of Blood in Shock and Hemorrhage, *ibid.*, p. 526.
- <sup>3</sup>Cannon, W. B., France: Acidosis in Cases of Shock, Hemorrhage and Gas Infection, *ibid.*, p. 531.
- <sup>4</sup>Cowell, E. M., France: The Initiation of Wound Shock, *ibid.*, p. 611.
- <sup>5</sup>Cannon, W. B., France: A Consideration of the Nature of Wound Shock, *ibid.*, p. 611.
- <sup>6</sup>Cannon, W. B., Fraser, John, and Cowell, E. M., France: The Preventive Treatment of Wound Shock, *ibid.*, p. 618.
- <sup>7</sup>Henderson, Y., and Haggard, H. W.: Respiratory Regulation of the  $CO_2$  Capacity of the Blood, Jour. Biol. Chem., 1918, xxxiii, 345.
- <sup>8</sup>Scott, F. H.: Factors Influencing the Interchange of Fluid between Blood and Tissue Spaces. I. Blood Pressure, Am. Jour. Physiol., 1917, xlv, 298.
- <sup>9</sup>Van Slyke, D. D.: Jour. Biol. Chem., 1917, xxx, 347.

—J. J. R. M.

# *The Journal of Laboratory and Clinical Medicine*

VOL. III.

ST. LOUIS, JUNE, 1918

No. 9

## ORIGINAL ARTICLES

---

### RESEARCHES IN RHEUMATISM\*

BY WILLIAM LINTZ, M.D., BROOKLYN, N. Y.

ANY work which will shed some light as to the etiology of rheumatism is very desirable since our knowledge as to the definite cause of this widespread disease is still in darkness. With this object in view I have investigated the blood of a great many cases of rheumatism, particularly of the acute articular type, but in only four cases was I successful in isolating microorganisms in the blood. Why the percentage of positive findings is so small is as yet not clear to me, unless the bacteria are lodged within Aschoff's nodes as I have described previously.†

The object of this work is

1. To demonstrate that in at least a small percentage of cases of acute articular rheumatism a definite microorganism can be isolated from the blood.
2. To describe in detail the study of the bacteriology and biochemistry of the bacteria isolated.
3. By animal experimentation to establish the pathogenicity of the microorganism, and its relationship to rheumatism isolated.

Let us consider these in the order mentioned.

1. A brief summary as to the different views elaborated to explain the causes of rheumatism is but fair in order to bring out all the phases of this important question.

A. Rheumatism was attributed by Cullen to be caused by cold acting directly upon the joints thus setting up a local inflammation, which is followed by a general fever. Exposure thus causing an irritation of the cutaneous sensory nerve fibers would set up a central disturbance in the cord and medulla oblongata.

\*From Hoagland Laboratory of Long Island College Hospital, Brooklyn, N. Y.

†Rheumatic Carditis, Jour. Am. Med. Assn., March 2, 1912.

This disturbance was transmitted to the nerves of the various organs which were involved by acute rheumatism and caused in turn the various manifestations of the disease.

B. Chemical theory: Both uric and lactic acids are supposed to be the cause, for they have been demonstrated in excess in patients suffering from rheumatism.

C. Infectious theory: This is viewed from various angles.

(1) Some believe there is no specific bacteria, but that the disease is a form of septicemia caused by staphylococcal or streptococcal infection.

(2) Some, that the disease is due to a specific bacillus (Achalme, 1891).

(3) Some, that the microorganism is a specific diplococcus. (Discovered by Triboulet in 1897.)

(4) That the microorganism has as yet not been discovered.

It seems to me that the first view (A) hardly needs to be considered, for it is entirely dispelled by the light shed by modern pathology. Such a pathogenesis would be indeed unique. The second view (B) hardly deserves more serious attention. For it is one thing to demonstrate an excessive formation of such substances, and another to prove them the cause and not the result of the disease. Besides, what one chemical substance will satisfactorily account for all the phenomena of rheumatism?

There is no doubt that the disease is caused by a microorganism, for the following reasons:

Sometimes this disease occurs in epidemics, as described by Strümpell in Leipzig, by Large in Copenhagen, and several have been observed in London.

The disease is self limited.

The severer symptoms and complications suggest those of other infections, some like pyemia, gonorrhea, scarlatina, typhoid fever, and cerebrospinal meningitis, may present joint symptoms.

All the experimental evidences by numerous workers go to the support of the bacterial cause of the disease. To me the report of James M. Beattie\* is very significant towards proving the infectiousness of rheumatism. He reports four cases of typical subacute articular rheumatism of long standing which presented amyloid degeneration of the organs. There was no other condition clinical or pathologic to account for this amyloid degeneration except rheumatism. Though it is true that the cause of amyloid degeneration is not clearly understood, yet it is generally regarded that it is secondary to some infectious agent, and that most generally bacterial in nature.

2. The four cases which yielded positive blood cultures were typical cases of acute articular rheumatism. Here the correct diagnosis is of the utmost importance. I will therefore cite the history of one of these cases, the other three cases presented practically the same symptoms and ran the same course. To cite them in detail would be merely a question of repetition.

Case P. L., Hospital No. 4644, was admitted to the Brooklyn Jewish Hospital on Jan. 1, 1910. His family history, as well as previous and venereal histories were negative, and had no bearing upon the present illness. His habits were good. His present illness began

\**Jour. Exper. Med.*, March 4, 1907.



[illegible]

TABLE II  
AEROBIC GROWTHS, 24-HOUR CULTURES AT 37.5° C.

	A	B	C	D
I	<p>AGAR</p> <p>Very profuse growth, colonies extremely minute, almost microscopic, grayish white in appearance, round edges, homogeneous, slightly raised, discrete although occasionally confluent. Edge of growth granular in appearance due to discrete colonies. Water of condensation clear. No change in media. 48 hr. growth no change.</p> <p><i>Microscopic</i>—Gram-positive diplococci.</p> <p>Growth more profuse, colonies more confluent, somewhat larger, on point.</p> <p>Otherwise same as above. Heavy growth in water of condensation.</p> <p>48 hour growth—more profuse, cloudiness and uniform precipitation of media.</p> <p><i>Microscopic</i>—Gram-positive diplococcus, 1 micron in diameter.</p>	<p>Same as A.</p> <p>Gram-positive diplococci</p> <p>Same as A.</p> <p>48 hr. growth, slight cloudiness of media.</p> <p>Same.</p> <p>Same as A.</p> <p>48 hr. slight cloudiness of media.</p> <p>Same.</p> <p>Same as A.</p> <p>48 hr. same as A.</p> <p>Same as A.</p>	<p>Growth moderate, glistening, colonies are raised, round, convex <math>\frac{1}{10}</math> to <math>\frac{1}{32}</math> mm. in diameter, media is unchanged, and water of condensation is perfectly clear.</p> <p>Growth moderate, otherwise same as above.</p> <p>Growth unchanged after 72 hr.</p> <p>Slight growth along stab for <math>\frac{1}{16}</math>" from surface, grayish white in appearance.</p> <p>72 hr. no change, growth scanty, and limited to needle track. No gas formation.</p> <p>Grayish white discrete colonies, round, elevated, size of "period dot." Growth moderate, heavy growth in water of condensation. No liquefaction of media.</p> <p>72 hr. growth more abundant, otherwise unchanged.</p> <p>Moderate growth same as IV.</p>	<p>Same as C, except that there is a slight granular precipitate in liquor condensation.</p> <p>Growth opaque, abundant, glistening; colonies round, convex <math>\frac{1}{10}</math> to <math>\frac{1}{4}</math> mm. in diameter. Liquor condensation turbid, with marked granular sediment.</p> <p>Same as C.</p> <p>Same as C.</p> <p>Same as C.</p> <p>Same as C.</p>
II	<p>GLUCOSE AGAR (SLANT)</p> <p>Growth more profuse, colonies more confluent, somewhat larger, on point.</p> <p>Otherwise same as above. Heavy growth in water of condensation.</p> <p>48 hour growth—more profuse, cloudiness and uniform precipitation of media.</p> <p><i>Microscopic</i>—Gram-positive diplococcus, 1 micron in diameter.</p>	<p>Same as A.</p> <p>Gram-positive diplococci</p> <p>Same as A.</p> <p>48 hr. growth, slight cloudiness of media.</p> <p>Same.</p> <p>Same as A.</p> <p>48 hr. same as A.</p> <p>Same as A.</p>	<p>Growth moderate, otherwise same as above.</p> <p>Growth unchanged after 72 hr.</p> <p>Slight growth along stab for <math>\frac{1}{16}</math>" from surface, grayish white in appearance.</p> <p>72 hr. no change, growth scanty, and limited to needle track. No gas formation.</p> <p>Grayish white discrete colonies, round, elevated, size of "period dot." Growth moderate, heavy growth in water of condensation. No liquefaction of media.</p> <p>72 hr. growth more abundant, otherwise unchanged.</p> <p>Moderate growth same as IV.</p>	<p>Same as C, except that there is a slight granular precipitate in liquor condensation.</p> <p>Growth opaque, abundant, glistening; colonies round, convex <math>\frac{1}{10}</math> to <math>\frac{1}{4}</math> mm. in diameter. Liquor condensation turbid, with marked granular sediment.</p> <p>Same as C.</p> <p>Same as C.</p> <p>Same as C.</p> <p>Same as C.</p>
III	<p>GLUCOSE AGAR (STAB)</p> <p>Profuse growth limited to needle track, no branching out into media.</p> <p>Growth homogeneous throughout, i. e., lower half just as thick as upper half. No gas formation. Colonies same as on I.</p> <p>48 hr. growth uniform, cloudiness, and precipitation of media; no gas formation.</p> <p><i>Microscopic</i>—Gram-positive diplococci, 1 micron in diameter.</p>	<p>Same as A.</p> <p>Gram-positive diplococci</p> <p>Same as A.</p> <p>48 hr. growth, slight cloudiness of media.</p> <p>Same.</p> <p>Same as A.</p> <p>48 hr. same as A.</p> <p>Same as A.</p>	<p>Growth moderate, otherwise same as above.</p> <p>Growth unchanged after 72 hr.</p> <p>Slight growth along stab for <math>\frac{1}{16}</math>" from surface, grayish white in appearance.</p> <p>72 hr. no change, growth scanty, and limited to needle track. No gas formation.</p> <p>Grayish white discrete colonies, round, elevated, size of "period dot." Growth moderate, heavy growth in water of condensation. No liquefaction of media.</p> <p>72 hr. growth more abundant, otherwise unchanged.</p> <p>Moderate growth same as IV.</p>	<p>Same as C, except that there is a slight granular precipitate in liquor condensation.</p> <p>Growth opaque, abundant, glistening; colonies round, convex <math>\frac{1}{10}</math> to <math>\frac{1}{4}</math> mm. in diameter. Liquor condensation turbid, with marked granular sediment.</p> <p>Same as C.</p> <p>Same as C.</p> <p>Same as C.</p> <p>Same as C.</p>
IV	<p>SERUM AGAR</p> <p>Very profuse growth having same characteristics as I. No liquefaction of media. Heavy growth in water of condensation.</p> <p>48 hr. growth more profuse.</p> <p><i>Microscopic</i>—Gram-positive diplococci and short chains.</p>	<p>Same as A.</p> <p>Gram-positive diplococci</p> <p>Same as A.</p> <p>48 hr. growth, slight cloudiness of media.</p> <p>Same.</p> <p>Same as A.</p> <p>48 hr. same as A.</p> <p>Same as A.</p>	<p>Growth moderate, otherwise same as above.</p> <p>Growth unchanged after 72 hr.</p> <p>Slight growth along stab for <math>\frac{1}{16}</math>" from surface, grayish white in appearance.</p> <p>72 hr. no change, growth scanty, and limited to needle track. No gas formation.</p> <p>Grayish white discrete colonies, round, elevated, size of "period dot." Growth moderate, heavy growth in water of condensation. No liquefaction of media.</p> <p>72 hr. growth more abundant, otherwise unchanged.</p> <p>Moderate growth same as IV.</p>	<p>Same as C, except that there is a slight granular precipitate in liquor condensation.</p> <p>Growth opaque, abundant, glistening; colonies round, convex <math>\frac{1}{10}</math> to <math>\frac{1}{4}</math> mm. in diameter. Liquor condensation turbid, with marked granular sediment.</p> <p>Same as C.</p> <p>Same as C.</p> <p>Same as C.</p> <p>Same as C.</p>
V	<p>GLUCOSE SERUM AGAR</p> <p>Extremely profuse growth. Marked clouding and precipitation of media. Surface granular. Colonies white or milky gray, round and size of "period." Thick and flocculent growth in water of condensation. No gas formation. There is marked precipitation of media giving it a milky color. No liquefaction of media.</p> <p>48 hr. growth more profuse and more precipitation of media. (This is optimum media.)</p> <p><i>Microscopic</i>—Gram-positive diplococci and short chains.</p>	<p>Same as A.</p> <p>Gram-positive diplococci</p> <p>Same as A.</p> <p>48 hr. growth, slight cloudiness of media.</p> <p>Same.</p> <p>Same as A.</p> <p>48 hr. same as A.</p> <p>Same as A.</p>	<p>Growth moderate, otherwise same as above.</p> <p>Growth unchanged after 72 hr.</p> <p>Slight growth along stab for <math>\frac{1}{16}</math>" from surface, grayish white in appearance.</p> <p>72 hr. no change, growth scanty, and limited to needle track. No gas formation.</p> <p>Grayish white discrete colonies, round, elevated, size of "period dot." Growth moderate, heavy growth in water of condensation. No liquefaction of media.</p> <p>72 hr. growth more abundant, otherwise unchanged.</p> <p>Moderate growth same as IV.</p>	<p>Same as C, except that there is a slight granular precipitate in liquor condensation.</p> <p>Growth opaque, abundant, glistening; colonies round, convex <math>\frac{1}{10}</math> to <math>\frac{1}{4}</math> mm. in diameter. Liquor condensation turbid, with marked granular sediment.</p> <p>Same as C.</p> <p>Same as C.</p> <p>Same as C.</p> <p>Same as C.</p>
VI	<p>GLYCERIN AGAR</p> <p>Growth sparse, hardly perceptible with naked eye, colorless and translucent. Colonies are microscopic, discrete. No cloudiness of water of condensation.</p> <p>48 hr. meager growth, colonies microscopic.</p> <p>Gram-positive diplococci and short chains.</p>	<p>Same as A.</p> <p>Gram-positive diplococci</p> <p>Same as A.</p> <p>48 hr. growth, slight cloudiness of media.</p> <p>Same.</p> <p>Same as A.</p> <p>48 hr. same as A.</p> <p>Same as A.</p>	<p>Growth moderate, otherwise same as above.</p> <p>Growth unchanged after 72 hr.</p> <p>Slight growth along stab for <math>\frac{1}{16}</math>" from surface, grayish white in appearance.</p> <p>72 hr. no change, growth scanty, and limited to needle track. No gas formation.</p> <p>Grayish white discrete colonies, round, elevated, size of "period dot." Growth moderate, heavy growth in water of condensation. No liquefaction of media.</p> <p>72 hr. growth more abundant, otherwise unchanged.</p> <p>Moderate growth same as IV.</p>	<p>Same as C, except that there is a slight granular precipitate in liquor condensation.</p> <p>Growth opaque, abundant, glistening; colonies round, convex <math>\frac{1}{10}</math> to <math>\frac{1}{4}</math> mm. in diameter. Liquor condensation turbid, with marked granular sediment.</p> <p>Same as C.</p> <p>Same as C.</p> <p>Same as C.</p> <p>Same as C.</p>

TABLE II—Cont'd  
AEROBIC GROWTHS, 24-HOUR CULTURES AT 37.5° C.

	A	B	C	D
VII	GLYCERIN SERUM AGAR	Growth very sparse, almost imperceptible. Few microscopic translucent colonies. Water of condensation clear and no liquefaction of media. 48 hr. fairly profuse growth of microscopic, translucent, discrete and confluent colonies. Growth in water of condensation. Gram-positive diplococci and short chains.	No growth.	No growth.
VIII	INULIN	No change, media not fermented. 48 hr.—no change. <i>Microscopic</i> —Diplococci.	48 hr. no growth. Same. Fermentation present.	48 hr. No growth. Same. Slight fermentation.
IX	LITMUS MILK	Acid, no coagulation, white precipitate at bottom.	Diplococci. Marked acidity. Coagulation.	Diplococci. Same as C.
X	LUNHAM'S PEPTONE	48 hr. acid, coagulated (test tube can be inverted without spilling contents). <i>Microscopic</i> —Gram-positive diplococci and short chains. Clear, no growth present. 48 hr. slight cloudiness and precipitate at bottom. <i>Microscopic</i> —No bacteria.	48 hr. white, and culture solid. Same. Same as B.	72 hr. solidification complete. Same. Same as B.
XI	ASCITIC SERUM	No change. No cloudiness.	Same as B.	Same as B.
XII	PLAIN BULLION	48 hr. no change. <i>Microscopic</i> —Short chains of cocci, some swollen, irregular and acicular in shape. Degeneration form. Uniform cloudiness, no ppt. 48 hr. same. <i>Microscopic</i> —Pairs and short chains.	Same as B. Same as A. Same.	Same as B. Same as A.
XIII	GLUCOSE BULLION	Moderate uniform cloudiness of media, due to suspension of fine granules, which are grayish white in color varying in size from minute pin points to "period dots." Heavy grayish white granular ppt. at bottom. 48 hr. growth more profuse. <i>Microscopic</i> —Pairs and short chains and in clumps resembling "amblyoboccus"	Same as A. 48 hr. same as A. Very long and tortuous chains of cocci. Same as A.	Same as A. Mainly as diplococci. Short chains.
XIV	BLOOD SERUM AGAR	Very profuse growth, edges of growth rough, due to discrete colonies. Colonies minute pin points, round and slightly elevated. No hemolysis, no liquefaction of media. 48 hr. growth more profuse, no hemolysis present. Gram-positive diplococci and short chains.	Profuse growth, pin-point colonies. Slight hemolysis. Otherwise same as A and B. Same.	Same as A. Same.

TABLE II—CONT'D  
AEROBIC GROWTHS, 24-HOUR CULTURES AT 37.5° C.

	A	B	C	D
XV	GELATINE AT ROOM TEMP. 75° F. POTATO	Same as A. Same as A. Same. Same.	Very fine growth. No liquefac- tion. 48 hr. same. Same. No growth.	Same as C. Same. No growth.
XVI	Profuse growth only along stab. Colonies are very small and grayish white. No liquefaction of media. 48 hr. more profuse growth and beginning liquefaction. <i>Microscopic</i> —Gram-positive diplococci. Moderate colorless or slightly grayish growth. No change in media. No odor. <i>Microscopic</i> —Short chains.	Same but more sediment. Moderately long chains. Same. Diplococci.	Diffuse granular turbidity. No fermentation. Diplococci. Slight turbidity. No fermentation. Diplococci.	Slight turbidity. No fermentation. Diplococci. Diffuse granular turbidity. No fermentation. Diplococci.
XVII	Profuse growth throughout media; no gas formation; no sediment. <i>Microscopic</i> —Diplococci and few short chains.	Same. Same.	Same.	Same.
XVIII	Very profuse growth. Heavy grayish white sediment. No gas formation. Growth thick and heavy near open end of fermentation tube, closed end almost clear. <i>Microscopic</i> —Diplococci.	Same. Diplococci.	Same.	Same.
XIX	Profuse growth more heavy near open part of fermentation tube. Profuse grayish white granular sediment. No gas formation. <i>Microscopic</i> —Mostly in clumps, here and there in diplococci and short chains. Very profuse growth same as XIX.	Same. Same. Same. Same.	Uniform turbidity. Same. Same.	Slight uniform turbidity. Cocci microscopically. Same.
XX	<i>Microscopic</i> —Gram-positive diplococci and short chains. Very profuse growth and uniform cloudiness of media. No sediment (same as XVI). No fermentation.	Same. No gas. Cloudiness of media more uniform. Same. Same.	Same.	Same.
XXI	<i>Microscopic</i> —Mostly in clumps; few diplococci. Diffuse uniform cloudiness. Slight grayish white sediment. No fermentation. <i>Microscopic</i> —Mostly in clumps and diplococci.	Same. Long chains. Slight turbidity, no fermentation. Same as A.	Same as A.	Same as A.
XXII	Slight turbidity. No fermentation. No fermentation.	Same.	Same as A.	Same as A.
XXIII	Growth slight convex and glistening. Media salts apparently precipitated out. Colonies round, entire, convex, about 1/4 mm. in diameter. Takes at least 2 to 3 days before growth appears. <i>Microscopic</i> —Gram-positive diplococci, some in short chains.	Same.	Gram-positive diplococci and involution forms present.	Same as A.
XXIV	Prowed. Growth very abundant, discrete, glistening. Colonies round, entire, discrete, convex, varying in size from .1 to .4 mm. in diameter. <i>Microscopic</i> —Gram-positive diplococci chains about 4, tending toward lanceolot shape at times.	Same. Same, containing gram-negative cocci.	Same. Same, containing cocci more lanceolot.	Same as A.



morphology, biology, biochemistry, and pathogenicity of the bacteria isolated was carefully studied, and comparisons were made under identical conditions with known streptococci isolated from various sources, other than rheumatism.

The results can be briefly represented in the Tables. The letters *A*, *B*, *C*, *D*, represent respectively the four strains of bacteria isolated from typical cases of rheumatism. *X*, *Y*, *Z* represent ordinary streptococci isolated by the author from sources other than rheumatism. *X* was isolated from a case of mastoiditis. *Y* was isolated from a case of pelvic abscess. *Z* was isolated from an ordinary abscess.

It is evident that these pathologic conditions can not possibly be considered as a form of rheumatism, and this is the reason why bacteria from these sources were chosen for comparison.

#### MORPHOLOGY

It is evident that from the point of view of morphology the above rheumatic strains represent nothing characteristic, the only difference being that in the rheumatic strains the cocci are smaller, and the chains are shorter.

#### CULTURAL CHARACTERISTICS

The bacteria were inoculated on the various media and studied both under aerobic and anaerobic conditions. The temperature of incubation was 37.5° C. The reaction of the media employed was 0.9 per cent acid.

A study under low power objective 16 mm., Ocular 4, of colonies, *aerobically* grown 24 hours at 37.5° C. on agar plates in streaks showed the following:

*Macroscopically*.—Colonies were small 1/10 to 1/2 mm., discrete, convex, raised, glistening, round, following line of streak.

*Microscopically*.—Round, edge entire, but faint, granules very coarse in center of colonies but getting fine towards edge. In center granules clumped together giving nucleated appearance, from which joint granules branch out in branches or strings towards the periphery.

After six days the growth was more profuse but of the same character. Litmus milk of *C* readily formed acid but only coagulated of three days.

#### ANAEROBIC CULTURAL CHARACTERISTICS AND MORPHOLOGIC STUDIES OF STRAINS *A*, *B*, *C*, *D*

The method employed was that of Buchner, the media exactly the same as for the aerobic cultures, the incubator temperature 37.5° C.

The bacteria grew almost as readily under anaerobic as under aerobic conditions. The growth however was a little slower and not quite so profuse. The bacteria are therefore facultative anaerobes. The anaerobic cultural characteristics are identical with those of the aerobic, and it will therefore be unnecessary to repeat the description. Morphologic studies of all cultures revealed gram-positive diplococci with tendency towards short chain formation.

Streptococci strains *X*, *Y*, and *Z* were cultured both aerobic and anaerobic under exactly the same conditions, and on the same media as strains *A*, *B*, *C*, and *D*. Strains *X*, *Y* and *Z* exhibited the usual cultural characteristics of

ordinary streptococci, and I therefore deem a detailed description of these unnecessary.

#### MORPHOLOGIC AND BIOLOGICAL DIFFERENCES BETWEEN STRAINS A, B, C, D, AND X, Y, Z

The main difference between the various strains of bacteria isolated from cases of rheumatism and the streptococci X, Y, Z were that the former were smaller and appeared mainly as diplococci with a slight tendency toward chain formation. On the other hand, the streptococci invariably appeared in long chains. Strain B appeared in long chains when grown on glucose bouillon, mannit, and salicin. On inulin, strains C and D caused fermentation (D only very slightly), A and B did not. None of the streptococci fermented inulin. The strains A, B, C, D grew *much more* rapidly and profusely than did X, Y, and Z. Under anaerobic conditions this contrast was even greater. Whereas the rheumatic strains grew almost as well under anaerobic conditions as under aerobic, the streptococci on the other hand, grew but sparsely and the majority of the cultures presented no growth at all. On litmus milk the contrast was marked, the rheumatism strains acidified and coagulated the media within 24 hours. You could turn the test tube upside down. The acid formation was very marked. On the other hand the streptococci X, Y, Z turned the blue litmus red but slightly and did not coagulate the milk. The acid formed was but slight. The changes in McConkey's media produced by the micrococcus rheumaticus is regarded as characteristic by Beattie.

The streptococci X, Y, Z would not grow upon this media.

#### BIOCHEMISTRY OF STRAINS A, B, C, AND D

The biochemistry of these microorganisms was extensively investigated for me by Dr. Leopold Rein. A summary of his conclusions to date was the following:

1. Absence of lactic acid in all cultures.
2. All bouillon media tubes and bouillon culture tubes give the same findings; viz., the acidity in terms of formic acid as 0.1.

#### DRYING EXPERIMENTS

These were conducted only with strain A of the rheumatism series, and X, Y, and Z of the streptococcus series. On June 3, 1910, a slant agar culture of Strain A was left at room temperature. The test tube was simply plugged with the ordinary cotton plug. By Dec. 1, 1910, all the agar in the test tube had dried up and a mere yellowish streak at the inside of the test tube was all that was left of the culture. I attempted to make spreads of the culture on glass slides but this was found physically impossible on account of the dryness of the media. I then poured some sterile plain bouillon into the test tube containing the dried culture and incubated at 37.5° C. At the end of 24 hours, to my great surprise, there was a profuse growth of gram-positive diplococci which had all the characteristics of the original strain A. This microorganism was

still viable after drying for six months. I repeated this drying experiment and found the microorganism viable after drying for 11½ months. For a nonspore-bearing microorganism this resistance to drying is certainly unique. The streptococci X, Y, and Z, on the other hand would die unless transplanted every four to seven days.

## ANIMAL EXPERIMENTATION

EXPERIMENT 1.—The microorganisms were grown on glucose agar and a saline emulsion was made of a 24-hour profuse growth, and injected intraperitoneally into *white rats*. The results can be best expressed by Table III

TABLE III

BACTERIAL STRAIN INOCULATED	<i>Rheumatic Strains</i>				<i>Ordinary Streptococcic Strains</i>		
	A RAT 1	B RAT 2	C RAT 3	D RAT 4	X RAT 5	Y RAT 6	Z RAT 7
Condition of rat							
8/5/10	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8/6/10	"	"	"	"	"	"	"
8/7/10	"	"	"	"	"	"	"
8/9/10	"	"	"	"	"	"	"
8/10/10	"	"	"	"	"	"	"

*Result.*—The rats were observed very carefully for 25 days and found absolutely normal. Neither the rheumatic nor the streptococcic strains had any effect on the rats.

## EXPERIMENT 2—

(a) Animals employed, 7 rabbits.

(b) Inoculation, intravenous.

(c) Cultures, saline emulsion of two 24-hour glucose agar growth.

(d) Date of inoculation, Aug. 10, 1910.

TABLE IV

BACTERIAL STRAIN	<i>Rheumatic</i>				<i>Streptococcic</i>		
	A RABBIT 1	B RABBIT 2	C RABBIT 3	D RABBIT 4	X RABBIT 5	Y RABBIT 6	Z RABBIT 7
Condition of rabbit							
8/10/10	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8/11/10	"	"	"	"	"	"	"
8/12/10	"	"	"	"	"	"	"
8/13/10	"	"	"	"	"	"	"
8/14/10	"	"	"	"	"	"	"
8/15/10	"	"	"	"	"	"	"
8/18/10	"	"	"	"	"	"	"
8/20/10	"	"	"	"	"	"	"
8/22/10	"	"	"	"	"	"	"
8/25/10	"	"	"	"	"	"	"

*Result.*—The rabbits were carefully observed for 20 days and all were found absolutely normal.

## EXPERIMENT 3.—

(a) Animals employed, 7 guinea pigs.

(b) Inoculation, intraperitoneal.

(c) Culture, 24-hour glucose bouillon.

(d) Date of inoculation, Aug. 17, 1910.

TABLE V

BACTERIAL STRAIN CONDITION OF GUINEA PIG	A GUINEA PIG 1	B GUINEA PIG 2	C GUINEA PIG 3	D GUINEA PIG 4	X GUINEA PIG 5	Y GUINEA PIG 6	Z GUINEA PIG 7
8/17/10	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8/18/10	"	Dead	"	"	"	"	"
8/19/10	"		"	"	"	"	"
8/19/10	"		"	"	"	"	"
8/20/10	"		"	"	"	"	"
8/21/10	"		"	"	"	"	"
8/22/10	"		"	"	"	"	"
8/23/10	"		"	"	"	"	"

*Result.*—The guinea pigs were observed for 25 days and found to be absolutely normal with the exception of guinea pig 2, which was inoculated with strain B. This pig was found dead 24 hours after the inoculation.

Autopsy of guinea pig 2 revealed a peritonitis which was caused by a Gram negative, nonmotile bacillus—contamination.

#### EXPERIMENT 4.—With strain A.

(a) Animal employed, young Newfoundland dog weighing about 80 to 90 pounds.

(b) Inoculation, intravenously.

(c) Culture, 24-hr. glucose bouillon culture.

(d) Date, Feb. 24, 1910, at 3 P.M.

Within 24 hours the dog developed stiffness and tenderness of the left knee joint. The next day the right knee joint became stiff and exquisitely tender. This stiffness and tenderness spread to the joints of the other extremities, so that in an attempt to walk the dog limped markedly. The joints felt hot. The animal was very thirsty, refused nourishment, perspired profusely and was very weak. His temperature ranged between 101-102° F. The dog was irritable and cried a great deal apparently from intense pain. This noise became so troublesome that it was necessary to kill him.

*Autopsy of Dog.*—On Feb. 28, 1910, at 4 P.M. dog was chloroformed. Autopsy revealed the following: All the large joints of the extremities were involved. The joints when opened showed a small amount of gelatinous, clear, viscid fluid and the blood vessels were injected.

The pericardium was inflamed and showed a large amount of fluid slightly tinged with blood.

The heart was otherwise normal and no lesions of the endocardium were demonstrable. The remaining organs revealed no pathologic changes.

#### *Spreads from*

(a) Joints showed gram-positive diplococci which had no capsules. There was also an occasional lymphocyte and polymorphonuclear leucocyte.

(b) Pericardial fluid—diplococci and streptococci and an occasional red blood cell.

#### *Cultures from*

(a) Blood of heart and veins showed short chains of streptococci.

(b) Pericardium—Streptococci of moderate and even long chains.

(c) Joints—Diplococci. The microorganism isolated showed in the main the same histologic characteristics of the original strain A with which the dog had been inoculated.

(d) Gram-positive diplococci were demonstrable within the tissues of the synovial membrane.

#### CONCLUSIONS

1. In some cases of acute articular rheumatism a microorganism can be isolated from the blood.

2. The reason that the positive blood cultures are not found more frequently in acute articular rheumatism is perhaps because the bacteria tend to



localize in Aschoff's nodules, and except in very virulent forms of the disease are rapidly destroyed in the circulation.

3. The microorganism isolated resembles a streptococcus.

4. Whether McConkey's bile salt media can be used as a differential media to exclude streptococci as claimed by Beattie must yet be proved. For we know that the streptococcus group is not only a large but a very variable one.

5. The resistance of the isolated microorganism to drying is certainly unique for a nonspore-bearing bacteria, particularly for a streptococcus.

6. Acute articular rheumatism, with its frequent complication, pericarditis, has apparently been reproduced in a dog, by an organism isolated from the blood of a patient suffering from a similar disease.

7. The reason why the other animals failed to contract the disease may have been due to

(a) Lack of susceptibility on the part of the animal.

(b) Attenuation of the microorganism or

(c) Both factors.

8. Morphologic identification of the microorganism is unreliable, since the diplococcus on some of the media will grow in long chains.

9. Further studies are necessary to determine whether acute articular rheumatism is caused by a specific microorganism, particularly immunologic investigations which are under way.

I have purposefully refrained from referring to the literature on this subject since my desire was to describe only my own personal experience.

The cases mentioned came from Dr. Merzbach's service at the Brooklyn Jewish Hospital.

---

## MASSIVE INFARCTION OF SPLEEN WITH REPORT OF A CASE\*

BY DEWAYNE G. RICHEY, PITTSBURGH, PA.

AT the autopsy table, infarction of the spleen is not infrequently encountered. The anatomical explanation of this appears to lie in the fact that the comparatively large splenic artery rapidly divides into numerous small terminal branches, possessing small lumina. The infarcted areas may be large or small, single or multiple. The multiple ones are usually small, although this does not necessarily obtain in all instances. Broadly speaking, however, splenic infarcts are larger than those found in other solid viscera, the reason for which again can be attributed to the manner of division of the radicals of the splenic vessels. When the infarcted area is small, it retains the wedge-shaped appearance, with the base directed toward the capsular surface. But if the infarct is quite large, say, involving more than one-fourth the organ, its typical shape is lost, and it becomes more irregular in outline, causing a subsequent distortion of the spleen. Moreover, splenic infarcts, as a rule, extend to the capsule of the

---

\*From the Magee Pathological Laboratories, Mercy Hospital, Pittsburgh, Pa.

organ, in contradistinction to some renal infarcts, for instance, which may be completely surrounded by living kidney tissue. The reason for this is, no doubt, that the capsular arterial distribution to the kidney does not find its analogue in the spleen.

Extensive infarcts of the spleen are by no means rare, yet it is unusual to have the entire organ incorporated in the infarcted zone. In recent years evidence has been produced which will readily account for this condition. Warthin called attention to the splenic anastomoses with the gastroepiploic vessels in the omentum gastrosplenicum, and later, Troell referred to an additional factor, that many broad, transverse anastomosing channels exist between the branches of both the splenic artery and vein, located close to the hilum of the organ. With this anatomical arrangement, it would necessitate either a very extensive blockage of the splenic blood channels or a gross anomaly associated with a lesser obstruction, to render virtually the entire spleen one huge infarct.

When massive infarction of the spleen does not occur, even the bland variety, if the condition is not recognized clinically and removed by operation, the result may be rapidly fatal. The findings of an old infarct involving practically the entire spleen, having occasioned no discomfort to the patient, and having played no direct part in his death, is rather a unique condition. In view of the fact that there is a paucity of references to similar conditions in the literature, particularly when contrasted with the correspondingly many allusions to other splenic processes, it was deemed worthy to report the findings in such a case.

The following case was admitted to the Mercy Hospital, Pittsburgh, Pa., on Jan. 12, 1917. It was assigned to the service of Dr. T. S. Arbuthnot, to whom I am indebted for the clinical records:

R. C., male, age 35, Negro, laborer, Hosp. No. 4648-17. On admission the patient was in a semicomatose state, so that a history from him was not available. Unfortunately the social status of the patient was such that no additional data could be obtained, save for the knowledge of a long standing deafness, and that the onset of the present illness had begun one week previously.

It was with the greatest difficulty that the patient could be aroused from his semicomatose condition, and at no time was he able to orientate himself. He presented a marked generalized hypertonicity, associated with extreme retraction of the head and rigidity of the neck.

Considerable pain was occasioned by movement of the head and by pressure over the tip of the left mastoid process. Deafness was quite obvious. The eyes were fixed and staring; the pupils equal, dilated, slightly irregular in outline and static. Thoracic examination revealed no essential organic lesions in the lungs or in the heart.

The abdomen was distended, very tense and both recti were rigid. There was no evidence of fluid in the peritoneal cavity, the spleen was not palpable. All reflexes, both superficial and deep were greatly exaggerated, but equal. Kernig's sign was definitely positive. No paralyses could be elicited. The temperature ranged from 102° to 104° F.; the pulse from 120 to 150; the respirations from 28 to 46. The blood pressure fluctuated within normal limits, around 100 (S.B.P.) and 80 (D.B.P.) mm. Hg. The blood count was as follows: R.B.C., 4,900,000; W.B.C., 45,100; Hgb. 88 (Sahli). Stained smears exhibited a marked polycytosis, (95 per cent), with a corresponding decrease in the lymphocyte forms. Analysis of a catheterized specimen of urine was negative. On lumbar puncture a turbid, almost milky, spinal fluid, under definitely increased tension, was recovered. Pneumococcus, Type IV, was isolated from the culture.

Despite vigorous eliminative and supportive measures, the patient pursued a rapidly fatal course, dying within 18 hours after admission, the clinical diagnosis was pneumococcal meningitis.

An autopsy was performed two hours after death by Drs. W. W. G. Mac-lachlan and DeW. G. Richey.

To obviate the rehearsal of a lengthy protocol, only the outstanding features of the autopsy findings both macro- and microscopical will be mentioned. Particular attention, however, will be paid to the spleen.

The body was that of an adult male negro, measuring 161 cm. in length. The body was well developed and well nourished. The head was markedly retracted. The entire surface of the brain was covered by a diffuse, greenish, adherent exudate, particularly well marked in the sulci, and as abundant anteriorly as it was posteriorly. The basilar region of the brain was bathed in a greenish, purulent cerebrospinal fluid. All the sinuses of the dura were clear. The left middle ear and the mastoid cells were filled with a gelatinous, pink, rather soft granulation tissue. The lungs exhibited no areas of consolidation, fibroses, or calcification. The heart valves were clear, presenting no acute, or chronic lesions. There was a moderate degree of cloudy swelling of the heart, liver and kidneys. The aorta, pancreas, adrenals, bladder and testes revealed nothing unusual.

A cursory examination of the splenic fossa gave the impression that the spleen was entirely absent. It was found, however, in the left hypochondrium, being 3.5 cm. above the upper pole of the left adrenal, and was imbedded in a dense layer of adipose tissue. Its only abnormal attachment was to the left parietal peritoneum, by several dense adhesions. The left border of the great omentum which was curled upward, spread outward in a fan-shaped manner, to its insertion along the mesial surface of the organ. Unfortunately, the splenic vessels were not dissected at this time, nor was the lumen of the splenic artery probed. No accessory spleens, enlarged lymph glands, nor hemolymph nodes were encountered.

*Spleen.*—Weight 25 grams. Measured 4.8 x 2.2 x 1.8 cm. The spleen was exceedingly small and had completely lost its normal appearance, being devoid of any of the gross landmarks by which it could be identified as such. Its removal with the perisplenic omental tissues, which was accomplished only with difficulty, revealed a very unique structure. It consisted of a large, oval nodule, with a dull, yellowish surface, and was very hard in consistency. At one end of the nodule, there were three irregularly oval lobulated masses, varying in size from 1.2 by 0.9 by 0.4 to 1.7 by 1.2 by 0.9 cm. Beneath the thickened capsule, these presented a reddish gray appearance, which simulated somewhat the color of splenic tissue. At the opposite end of the central nodule was a thin, fin-shaped piece of compressed tissue, measuring 4 by 2 by 0.2 cm. The surface was smooth, and it presented a dull, dark red appearance, beneath which several arborescent trabeculations could be seen. This piece of tissue was of approximately the thickness of the diaphragm, and looked not unlike that structure, but also suggested a splenic origin, inasmuch as the cut surface revealed a dull, red, meaty appearance, in which the cut mouths of the blood vessels were clearly observed. The entire organ, then presented three separate and distinct parts—(1) the nodular, hard center, which was the largest part; (2) the lobulated, landlike nodules at the lower pole; (3) the flattened fin-shaped tissue at the opposite pole.

On section through the organ, the central nodule was seen to consist of a yellowish, necrotic material, through which numerous dense strands of fibrous tissue coursed in an irregular fashion. Here and there, tiny granules of calcified material could be made out. At one place near the center, a small oval island of reddish tissue occurred, which bore close resemblance to the tissue noted at one end of the specimen. This area measured on the cut surface, 6 by 2 mm.

The appearance of this mass suggested a process of necrosis, with subsequent fibrosis and calcification, following extensive infarction of the organ. The cut surface of the three nodules previously described presented a dull red, smooth, glistening appearance, the center of which was a stellate, white, fibrous core, from which radiated numerous fibrous trabeculae. These nodules communicated with each other and, because of this fact, although they closely resembled splenic tissue, they did not convey the impression of accessory spleens, but rather a portion of the organ which had survived by virtue of the adhesions which were present at this point.

Microscopic section of the spleen showed, along one border, a narrow strip of splenic tissue. This area revealed the usual picture of splenic pulp, except that there was a marked increase in the fibrous connective tissue. The capsule was markedly thickened, contained many small blood vessels. The sinuses of the splenic pulp were dilated and them could be seen erythrocytes, endothelial cells, lymphocytes and leucocytes. Of the

latter, the polymorphonuclears remained. Only vestiges of the lymphoid tissue of the organ remained. One small Malpighian corpuscle was noted. Immediately contiguous with this area, and sending broad, irregularly arborescent ramifications into it, could be seen a wide expanse of tissue, densely fibrous in appearance. This area was composed of variously-sized islands of hyaline material, which were supported by a dense fibrous stroma. In the section no evidence of calcification was present, though the character of the tissue was such that it might well have been expected. In curious cleftlike interstices in this solid fibrous tissue, masses of brown crystalline pigment in large masses, were noted. The large masses had provoked giant-cell formation, of the foreign body type, while smaller particles lay in endothelial cells. There was no evidence of tubercle formation. Cultures taken from the heart's blood and meninges, at autopsy, showed the pneumococcus; the culture from the mastoid showed, in addition to this organism, the staphylococcus pyogenes aureus, and *B. pseudo-influenzæ*. All the strains of pneumococcus which were isolated, fermented lactose, salicin and inulin, and belonged to Group IV.

The anatomical diagnosis can be condensed to the following: acute suppurative meningitis (pneumococcic); acute and chronic suppurative otitis media (left); acute and chronic suppurative mastoiditis (left); cholelithiasis; massive infarction of spleen; atrophy of spleen; chronic perisplenitis.

In summarizing the case, we have a male negro, 35 years of age. He was admitted to the hospital in a semicomatose state. The only available history was that of a protracted deafness and that the onset of his present illness had antedated his death by a period of eight days. There was no history, or antemortem physical or laboratory finding which pointed to the presence of any splenic condition. The cause of death was directly referable to an acute suppurative meningitis (pneumococcic), the origin of which was an old otitis media and mastoiditis. The spleen was very atrophic, showing a large area of necrosis, fibrosis and calcification, and two small peripheral masses of living splenic tissue surrounded by dense fibrous adhesions. The positive findings in the other viscera were coincidental to the acute infection of the meninges, save for the presence of many small gall-stones. No focus from which an embolus could have arisen was found. No congenital abnormalities were evident.

Absence of the spleen may be a congenital anomaly or the result of surgical intervention and is not incompatible with life. Hodenpyl, in reporting a case of apparent absence of this organ, was able to collect nine other cases from the literature. Others have cited instances where a congenital absence occurred in monsters, notably Hensinger and Potter. The three most common findings associated with splenic agenesis are accessory spleens, generalized lymphatic hyperplasia and hemolymph nodes. The accessory spleens may vary from one to fifty, as cited by Thorel, or 400, as mentioned in the case of Albrecht. The marked generalized lymphatic hyperplasia was one of the outstanding features of Hodenpyl's case. The occurrence of hemolymph nodes is probably closely analogous to this phenomenon, for, as Warthin has pointed out, these structures apparently simulate lymph-glands in function.

References to diminutive spleens are not commonly encountered in the literature. Calvert reports the case of such a spleen weighing 2.1 grams, which was removed at autopsy from a female 30 years of age. Microscopically, diminished splenic pulp, thickened arterial walls and trabeculae were the main considerations. Glinski found a small spleen at necropsy in a female, aged 4, who died of pulmonary tuberculosis. The condition, he intimates had no bearing on the size of the spleen, but offers no explanation regarding the etiology.



of the splenic finding. Paulesco records the case of "rudimentary spleen," measuring 5 by 1 by 2.6 cm.

Again, one encounters but few cases of extensive infarction of the spleen, with marked atrophy. Vintele apparently encountered such a case, but his article was not obtainable at this time. Nuzum, in a recent communication on infarction of the entire spleen, was able to collect twenty-eight cases in the literature, to which he added four of his own. In only two of the entire series of cases were the spleens small, and in only one, that of Durand, could the finding be compared with our case. In this instance the organ was described as a "gigantesque cicatrix." In all the other cases the spleen was enlarged, being in one case as heavy as 2700 grams and in another as large as 25 by 15 by 8 cm. There is but little doubt that the variations in the proportions of the organs in this series and ours could be adequately explained from the standpoint of time rather than the amount of involvement or the manner in which the infarction occurred. As Nuzum points out, the atrophic and fibrosed spleens represent the final stage in the process of repair of the infarcted area. It is imperative, if there be a culmination of the process in cicatrization, that the etiologic factor be of a sterile nature, and, but to a lesser extent, of a gradual onset. Rapid occlusion of both splenic vessels leads almost without exception to a fatal termination, if the causative agent is infective through abscess formation or through absorption of the toxic products of cellular disintegration, if the agent be sterile. Hence, progressive thrombosis of both the splenic artery and vein is the probable mode of development of the lesion in our case, for, as Nuzum's examples would indicate, an embolus of the splenic artery rarely causes infarction of the entire spleen.

The various stages through which a bland infarct passes has always been subject of great interest. Whether in its incipency, an infarct is hemorrhagic or anemic has called forth considerable debate among many ardent workers. Some writers, notably Beckman and Weigert, followed by von Recklinghausen, Thoma and Orth, held the latter view and Uhthoff, Talma, Beattie and Dickson, and others, accepted the former theory. In later years, Karsner and Austin, arrived at the same conclusions, following a series of experiments on dogs. In any event there seems to be but slight doubt that whether an early infarct is hemorrhagic or anemic depends largely on the organ involved. Thus, infarcts of the retina and kidney are usually anemic, those of the lungs and intestines are practically always hemorrhagic. When hemorrhage does occur, it is usually by a process of diapedesis rather than rhexis. Welch and Mallard held that a high venous pressure favors hemorrhage in the infarcted area while low arterial pressure opposes it. It is highly probable that the whole story is not told from the point of view of pressure in the circulatory channels. As Clami points out, another important factor must be taken into consideration, namely the susceptibility of certain tissues to circulatory unrest, with a rapid discharge of autolytic enzymes, and liberation of prothrombin, so that coagulation occurs before diapedesis of the erythrocytes can take place. It seems logical to conclude with MacCallum, that death of the tissue in the area of infarction is due to the anemia. In our case the presence of considerable blood pig-

ment would indicate that there had been a considerable extravasation of blood into the infarcted area.

A consideration of the minutiae of the specimen we have described, reveals no characteristics which differ from the usual end results of bland infarcts. Of far more interest is the persistence of some of the splenic tissue. A concomitant perisplenitis is the usual finding in splenic infarcts due to irritation of bacteria or tissue disintegration. In the earliest stages a fibrinous exudate occurs, which later becomes organized. The omentum becomes adherent to the splenic surface, or, if the process is sufficiently extensive, may envelop the entire organ. This fact was noted by Troell, who found the spleens of two guinea pigs markedly diminished in size after ligation of the splenic vessels, despite the fact that in both instances the organs were enveloped in omentum. From this observation he concludes that a "subsidiary collateral circulation through the spleen capsule could not be developed and thus compensate the stoppage of the blood supply through the spleen hilum." This same author cites the instance of some experimental work carried on by Garlow and Sheldon, who ligated the entire hilum in three dogs, all of which died. The latter worker subsequently followed Pirone's method of attaching the omentum to the spleen. Again, all the animals died. On the other hand, Jamison observed that, in ligating the splenic pedicle in five dogs and covering the spleen by omentum all the dogs recovered, there was no severe crisis, as seen in the cases where the omentum was not attached, and the degree of atrophy was greater. The results of these investigations are widely divergent. From the data on hand it would be difficult to draw any conclusions as to the function that the omentum plays in this type of case.

The author wishes to thank Doctor Oskar Klotz for many valuable suggestions during the preparation of this paper.

#### BIBLIOGRAPHY

- Adami and Nicholls: Principles of Pathology, 1911, ii, 42 and 222.  
 Albrecht: Ziegler's Beiträge, 1896, xx, 513.  
 Calvert: Am. Jour. Med. Sc., 1905, cxxx, 311.  
 Hodenpyl: Proc. New York Path. Soc., 1898, p. 185.  
 Karsner and Austin: Jour. Am. Med. Assn., 1911, lvii, 951.  
 MacCallum: Textbook of Pathology, 1916, p. 27.  
 Nuzum: Jour. Am. Med. Assn., 1918, lxx, 282.  
 Potter: Jour. Am. Med. Assn., 1906, xlvii, 363.  
 Roble: Jour. Am. Med. Assn., 1915, lxiv, 796.  
 Rokitansky: Path. Anat., 1899, ii, 165.  
 Sheldon: Am. Jour. Med. Sc., 1910, cxxxix, 581.  
 Thorel: Lubarsch-Ostertag's Ergebnisse d. allg. Path., (1900-1901), vii, 98.  
 Troell: Ann. Surg., 1916, lxiii, 88.  
 Warthin: Am. Jour. Anat., 1901, i, 63.  
 Warthin: Proc. Soc. Exper. Biol. and Med., 1907, iv, 127.  
 Welch: System of Medicine, Allbutt and Rolleston, 1909, vi, 781.

## THE ETIOLOGY OF SCARLET FEVER\*

### II. A SKIN TEST WHICH MAY INDICATE IMMUNITY TO SCARLET FEVER

By R. W. PRYER, D.P.H., AND GEORGE SEWELL M.D., DETROIT, MICH.

In a previous paper<sup>1</sup> from this department, mention was made of a large organism which was isolated from the blood in a fatal case of scarlet fever,—to summarize briefly:

This organism varies in length from 2 to 8 microns and seldom exceeds 4 microns in diameter. In young cultures the organism tends to be spherical in shape. It stains readily and tends to be gram-negative although its behavior to Gram's stain is not constant. With a differential stain such as Giemsa's, a red granule can be made out in a faintly staining central portion. This organism grows best on blood agar and cultures on this media give a peculiar distinctive color. The growth in liquid media is very slow, no pellicle is formed but a slimy sediment gradually collects. In sugar media no gas is formed and alkali is slowly produced. Gelatin is not liquefied.

It is not our intention in this paper to go deeper into the cultural characteristics of this organism but in the near future we expect to present an article on this subject and desire to state at this time that we have obtained this same organism from the throat of scarlet fever patients, although only in a very limited number of cases. Cantacuzene,<sup>2</sup> however, has described an organism similar to, but not identical with, the one described by us and states that while ordinary laboratory animals are immune to injections of these cultures, the injection into monkeys is followed by sickness and occasional death. The condition produced in monkeys is very similar to the clinical manifestations of scarlet fever in the human.

The skin test reported in this paper is based largely on the work of Gay<sup>3</sup> and his associates on the typhoidin test in typhoid fever. Typhoidin is prepared by evaporating a broth culture of *B. typhosus* at 56° C. to about 25 per cent of its original volume. This concentrated culture is then precipitated by slowly pouring into an excess of absolute alcohol. The precipitate is filtered off, washed in absolute alcohol, then with ether, dried, and finely pulverized in a mortar. The use of polyvalent preparation of typhoidin is recommended and the dose is given in the literature as 0.00002 gm.

It seems to us as though no definite dosage could be given for a preparation of this kind. We have found in our work that the dose of these preparations varies within wide limits and must be determined for each one just as it is necessary to determine the M.L.D. of each batch of diphtheria toxin and to carefully standardize each new antigen.

Typhoidin when injected intradermally into people who have had typhoid fever gives a distinct reaction in about 75 per cent of the individuals and this

\*From the Bureau of Laboratories of the Detroit Board of Health.

reaction persists for over forty-eight hours. In people who have been recently vaccinated against typhoid fever, the percentage of positive reaction is slightly lower, whereas in individuals with no history of typhoid fever, the percentage of positive reactions is only about 14.

Although we realize that we have not proved that this organism which for the sake of convenience we will refer to as B.B., is the cause of scarlet fever, we have called the preparations made from it scarlatin and in this paper we use that term to describe the material made from these cultures in a method similar to the manufacture of typhoidin from the typhoid bacillus.

The growth of the organism isolated by us is very slow in any liquid media consequently in order to get a heavy growth in broth considerable time is required. Our first scarlatin (Scarlatin 1) was prepared from a culture of B.B. No. 1, which had been growing in broth for eighty days. This preparation was accidentally destroyed before standardization was completed but gave results which encouraged us to proceed with this work.

Scarlatin No. 2 was also made from culture B.B. No. 1. The broth used was made from fresh beef and contained 2 per cent Difco peptone, 1 per cent glucose, 0.5 per cent salt and was adjusted to a final reaction of +1 by phenolphthalein. This flask was incubated for 70 days at 37° C.; at the end of this time the media had evaporated to about 25 per cent of its original volume. The growth of organisms was heavy and slimy and had settled to the bottom of the flask. The growth was proved to be pure by subculturing and microscopic examination. This growth was poured into 20 volumes of absolute alcohol stirring continually. The precipitate formed was too sticky to filter and was separated by centrifuging. The precipitate was washed with absolute alcohol then with ether by shaking in the tube, dried at 37° C. and then finely pulverized in a mortar.

Upon standardization of Scarlatin No. 2, 0.00002 gm. was found to be what we call the minimum effective dose; i. e., that amount which upon intradermal injection will give the highest number of positive reactions in scarlet fever convalescents and the lowest number in individuals with no history of scarlet fever. The solution for the test was made up so that this amount of weight of the power was contained in 1/10 c.c. of sterile physiologic salt solution. This amount was injected intradermally into scarlet fever convalescents and into people who gave no history of scarlet fever.

These injections were controlled by the use of a preparation of sterile broth evaporated to 25 per cent of the original volume and precipitated by absolute alcohol. The precipitate was washed with absolute alcohol and with ether, then dried and pulverized. The dose of this control has been the same amount as the scarlatin preparation and has been given into the other arm.

Table I shows the results obtained in a series of cases with Scarlatin No. 2. The patients with history of scarlet fever were patients in the scarlet fever pavilion of the Detroit Municipal Hospital and were in from the third to the fifth week of the disease. The control cases were patients who were in the same hospital but in different pavilions and were convalescent from other diseases. Most of these were about to be released after diphtheria.



TABLE I

	No.	+	?	-	% +
Scarlet fever	34	25	1	8	86
Not scarlet fever	35	4	3	28	11.5

These reactions were read at the end of twenty-four hours. A reaction is called positive when the area of inflammation is as large as a dime (about 18 mm. in diameter). Reactions recorded as questionable have a definite redness about the site of inoculation. Induration at site of inoculation without definite inflammation is recorded as negative.

This material appears to retain its potency indefinitely in the dry powdered state but when put in solution, deteriorates rapidly at room temperature. When kept in the ice box, the solution increases somewhat in strength at first and then gradually deteriorates.

Table II shows the results obtained with Scarlatin No. 2 on a few normal individuals:

TABLE II

	No.	+	-	% +
History of scarlet fever	2	2	0	100
No history of scarlet fever	4	2	2	50

It is our opinion that many people have had scarlet fever and are not aware of it. Farther on in this paper we will go deeper into this but we desire to state here that the two people with no history of scarlet fever who gave a positive reaction with Scarlatin No. 2 are much older than the other two. It is of course well known that older people are less liable to scarlet fever than children or young people.

In repeating this work a flask of media of the same composition but freshly made up was inoculated with the organism B.B. No. 1, and after three days in the incubator, this was concentrated and precipitated as before. This preparation was nearly inert, although by this time the organism was growing fairly well in liquid media. This preparation which we have labeled Scarlatin No. 3 is too weak.

Scarlatin No. 4 was made in the same way from a culture nine days old. This preparation, while not as strong as No. 2, seemed to give very good results. The following shows the standardization of this product.

In the first set of experiments 2 mg. of the powder was weighed out very carefully and then ground in a sterile mortar with 80 mg. of sodium chloride. In 10 c.c. of water was added in small portions, stirring after each addition. 10 c.c. of this solution was then injected intradermally.

Four scarlet fever patients and five others with no history of scarlet fever received this injection. None reacted.

In Table IV, the weight of scarlatin was increased to 5 mg. With a dose of 1/10 c.c., this is, of course, equivalent to .00005 gm. Again there were no reactions in either series.

The use of 10 mg. of powder with 80 mg. of salt in 10 c.c. of water has been the strength used in the following experiments. In most of these, a control injection has been made into the other arm, either of the same amount of culture media as heretofore described, or a typhoidin solution.

The typhoidin used was prepared from the Rawlins strain of *B. typhosus* according to the method described by Gay and his associates. We have not attempted to standardize this for the determination of immunity to typhoid fever, and all our reactions were read at twenty-four hours instead of the forty-eight-hour period recommended by workers with typhoidin.

Our object in using typhoidin in this work has been to see if scarlet fever left a hypersensitive condition of the skin. We do not believe that it does, but we have, we believe, shown that in the use of scarlatin on one arm controlled by an injection of typhoidin in the other, the reactions of each preparation are intensified and that such compared injections give misleading results.

In Table III the scarlatin used was No. 4 in 1/10 mg. dose. Part of these injections were controlled by an equal amount by weight of culture media control. Part were controlled by the use of typhoidin in 0.0000025 gm. doses. A few were not controlled at all. When a control was used it was always given intradermally into the right arm, the scarlatin being given in the left.

TABLE III  
SCARLATIN TESTS

	No.	+	?	-	% +
Scarlet fever convalescents 2nd to 5th week	42	22	2	18	52.5
No history of scarlet fever	31	5	2	24	16.1

In Table IV Scarlatin No. 4 in the same dose was used either alone or with culture media control. All controls in this series were negative.

TABLE IV

	No.	+	?	-	% +
Scarlet fever convalescents 2nd to 5th week	29	15	2	12	51.5
No history of scarlet fever	21	0	1	20	0

In Table V, the results with typhoidin are given in a series of cases. Part of these have had at the same time an injection of scarlatin in the other arm.

TABLE V

	No.	+	?	-	% +
Scarlet fever convalescents 2nd to 5th week	31	23	5	3	74
No history of scarlet fever	24	20	3	1	83.5

In Table VI the results with typhoidin alone are shown. These reactions were not controlled.

TABLE VI

	No.	+	?	-	% +
Scarlet fever convalescents 2nd to 5th week	13	9	2	2	69
No history of scarlet fever	14	11	2	1	79

Scarlatin No. 5 was prepared as follows: 100 c.c. 18-day broth culture of Organism No. I; 100 c.c. 18-day broth culture of Organism No. II and 100 c.c. 70-day broth culture of Organism No. II, were combined and evaporated at 56° C.

The 100 c.c. of broth in which Organism No. II had been growing for 8 days had comparatively few organisms in it while the others had a very heavy

growth. All cultures were subcultured and proved to be pure strains. Organism No. 1 was isolated from the blood, while Organism No. 2 was isolated from the throat of a scarlet fever patient.

Scarlatin No. 5 was prepared from this concentrate in the usual way and standardized; 0.00002 gms. was found to be the minimum effective dose. Table VII shows the results in a small series of tests at the Detroit Municipal Hospital.

TABLE VII

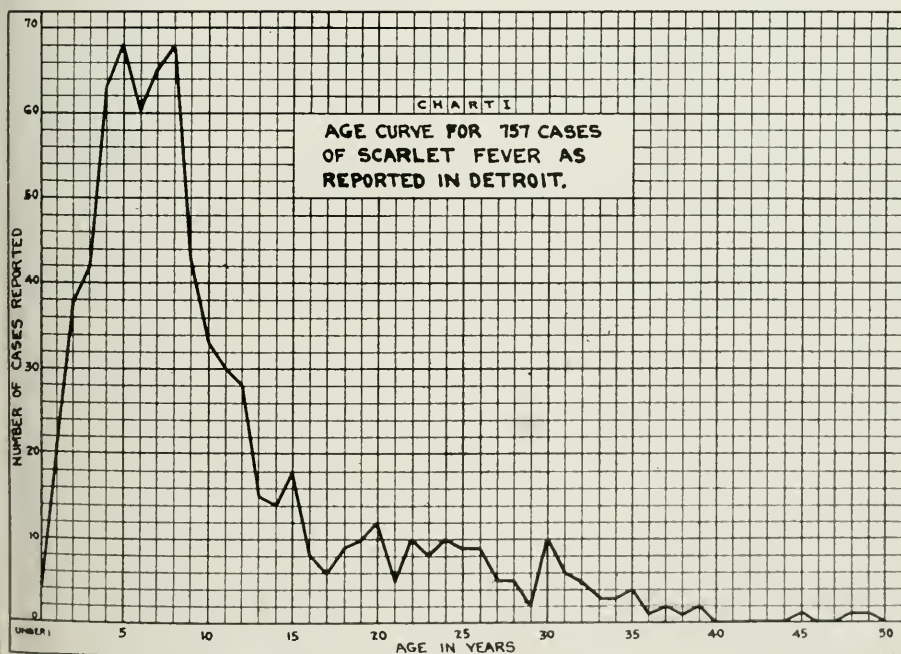
	No.	pos.	?	neg.
Scarlet fever 2nd to 5th week	35	15	14	6
No history of scarlet fever	20	3	3	14

Table VIII shows the results obtained in a series of tests with Scarlatin No. 5. The people included in this table were normal people with no infectious disease.

TABLE VIII

	No.	pos.	?	neg.
History of scarlet fever	12	7	3	2
No history of scarlet fever	25	5	6	14

It has been noted in this work that in people with no history of scarlet fever the probability of a positive reaction increases with age. Scarlet fever is essentially a disease of childhood. In Chart No. 1 the distribution by ages of the total number of cases of scarlet fever reported in Detroit between Nov. 1, 1917,



and Feb. 28, 1918, is shown graphically by the solid line. A study of this chart shows that of these 757 cases 609 or over 80 per cent are 15 years or less in age and that the highest incidence is between 5 and 8 years of age.

The number of tests on people with no history of scarlet fever is not as great as it should be. Taking those cases on which we have complete data we

have 83. Of these 83, 45 are less than 20 years of age and the reactions were recorded as, positive 6, questionable 5, negative 34. Of the 38 over 20 years of age our records show, 11 positive, 11 questionable, and 16 negative. In the cases with history of scarlet fever, the age did not influence the reaction in this way.

Table IX shows the results with scarlatin tests in scarlet fever convalescents arranged according to duration of the disease in weeks. This seems to show that immunity to scarlet fever is developed rather slowly, which is in agreement with the clinical experiences in this disease.

TABLE IX

Duration	No.	pos.	?	neg.	% +
2 weeks	11	4	0	7	36.3
2 to 4 weeks	25	11	8	6	44.
Over 4 weeks	25	14	5	6	56.

## SUMMARY

In a previous paper an organism of unusual characteristics was described. This organism was isolated from the blood of a man dying of scarlet fever. In this paper mention is made of the fact that we have found this same organism in the throat of scarlet fever patients, although only in a very low percentage of cases studied. Cantacuzene has described an organism found in the throat of scarlet fever patients which is evidently the same as the one described by us and has shown that this organism produces in monkeys a condition closely resembling scarlet fever.

We have shown that a preparation made from these cultures by a method similar to the preparation of typhoidin from the typhoid bacillus gives a higher percentage of reactions in scarlet fever convalescents than is the case in other infectious diseases, while typhoidin gives slightly more positive reaction in other infectious diseases than it does following scarlet fever.

The probability of a positive reaction with scarlatin, as we call this preparation, increases with the duration of disease and in people with no history of scarlet fever increases with age of the individual tested.

We do not hold that we have proved that this organism is the etiologic factor in scarlet fever, but we do believe that we have presented sufficient evidence to give this organism serious consideration as a possible and probable cause of the disease.

To better summarize our work, Table X shows our results with the different preparations in scarlet fever patients, in people with a past history of scarlet fever and in people with no history of scarlet fever infection.

TABLE X

	No.	pos.	?	neg.	% pos.	% ?	% neg.
Scarlet fever	125	71	20	34	56.7	16	27.3
No scarlet fever	115	19	14	82	16.5	12.3	71.2

The writers desire to express their appreciation to Dr. A. Lange for his assistance in portions of this work.

## BIBLIOGRAPHY

- <sup>1</sup>Pryer and Kelly: Jour. Lab. and Clin. Med., Feb., 1918, iii, 269.
- <sup>2</sup>Cantacuzene, M. J.: Compt. rend. Acad. d. sc., vol. clix, 381.
- <sup>3</sup>Gay, Force, and others: Jour. Lab. and Clin. Med., 1916, 1917.



## ADAPTATIONS OF RENAL FUNCTION TESTS FOR GENERAL USE

BY CAPT. WARREN T. VAUGHAN, M.R.C., U. S. ARMY\*

READERS of the current medical literature are receiving nowadays a vast accumulation of useful information concerning the more recent, so-called "tests of renal function." The technic of the various procedures has been pretty well perfected and the men who are especially interested in the study of the nephritis problem are now busy accumulating data concerning the reactions of these tests in the various types of nephritis and in other diseases. There is therefore today an abundant supply of information upon which we may rely in the interpretation of our results; and the conclusions drawn concerning the functional efficiency of an individual's kidneys are no longer hypothetical but may be based upon knowledge of scientific facts.

In most modern hospitals the "kidney tests" are performed on all cases in which the renal function is in question and some hospitals have gone so far as to make some of them, especially the "phthalein test," practically routine on all cases. A patient who enters a good hospital for medical observation has as thorough an examination of his kidneys as is made of his heart, lungs and digestive system.

Although the examination of a medical case for renal function is usually more satisfactorily conducted in a hospital, modifications of the newer methods may be easily adapted to an office practice. Alert practicing physicians have probably read most of the literature concerning the technic to be followed in hospital examinations, but they hesitate to modify it for office or home use because they are in no position to know how much the changes instituted are going to vitiate the results. The simplifications presented here are the result of an experience with the various tests as modified in the out-patient department of a large hospital.

For the sake of completeness certain rather elementary points will also be emphasized.

A really thorough examination of renal function presupposes in the first place a complete and thorough physical examination, including especially examination of the cardiovascular system, the throat and tonsils, the thyroid gland and the blood. In both hyper- and hypo-thyroidism, in primary or pernicious anemia and secondary anemias, as well as in certain other conditions, there is a demonstrable change in renal function, and it is important to recognize those conditions if present since they form a direct etiology for the kidney damage. Improvement in the condition of the kidneys will follow successful treatment of the other condition present. The frequent occurrence of chronic tonsillitis and other focal infections concomitant with chronic nephritis strongly suggests an infecting agent as a cause of renal damage, while microscopic examination of such kidneys frequently demonstrates the remains of metastatic foci in the

\*Base Hospital, Camp Sevier, Greenville, South Carolina.

organs themselves. All sources of local infection should therefore be sought out in the preliminary physical examination of a nephritis suspect. In the cardiovascular system there may be present left cardiac hypertrophy and a ringing aortic second sound, associated with a hypertension. Arteriosclerosis may accompany this picture or may be absent, or may even be present without hypertension. Every case with high blood pressure or arteriosclerosis, or both, should have careful studies of his renal function and in most cases there will be slight, but demonstrable, impairment, while in a few the damage will be very serious. Nephritis is the one particular disease in which the condition frequently progresses entirely insidiously and the patient does not consult the physician until certain symptoms, indicative of very severe kidney damage, finally appear and treatment will be of little or no avail.

OUTLINE FOR A COMPLETE EXAMINATION OF RENAL EFFICIENCY, APPLICABLE  
TO PRIVATE PRACTICE

(1) Make a complete routine *physical examination*. Map out the heart borders and note especially the distance of the left border and apex impulse from the mid line, their relation to the nipple line and in what interspace the maximum cardiac impulse lies. Note the character of the heart sounds, especially of the aortic second sound and observe the presence or absence of any murmurs at any of the valve areas.

The radial arteries should be palpated with a view to determining the presence or absence of thickening or of tortuosity. By bimanual palpation of one radial artery the presence or absence of any marked degree of hypertension may usually be determined. The index or middle finger of the left hand is used to exert pressure on the artery while the pulse is noted distal to the point of compression with the same fingers of the right hand. The compression force necessary to cause the pulse to disappear is the criterion sought. After some experience it is possible, as stated, to determine whether or not hypertension be present, but on the other hand, it is an extremely hazardous undertaking to try to estimate the *degree* of hypertension. The brachial and temporal arteries should also be examined for beading, tortuosity and thickening.

(2) *Take the blood pressure*, including both the systolic and diastolic pressures. In a very rough way the systolic pressure is an indication of the heart power and the diastolic indicates the condition of the vessels—of the peripheral resistance. Thus, in aortic insufficiency and hyperthyroidism, there frequently occur rather high systolic pressures with low or normal diastolic pressures. The high diastolic pressure is, therefore, frequently a better indicator of vascular or renal hypertension than is the systolic. A diastolic pressure persistently above 100 mm. Hg is pathologic, while one between 90 and 100 mm. Hg should arouse suspicion and is an indication for repeated examinations. As a rule a high diastolic pressure has associated with it an increased systolic pressure. A persistent systolic pressure of 160 mm. Hg in a young adult is undoubtedly abnormal. It is usually indicative of chronic nephritis and in the U. S. Army it is sufficient to disqualify for active service. It is highly essential to watch for abnormal variations in the blood pressure on different

occasions, particularly an increase of systolic pressure after exercise and excitement. It has been said that the normal average systolic rise after running fifty steps is 24 mm., the diastolic rise is about half as much or 12 mm. The lifting of a 20-pound dumb bell six feet thirty times in sixty seconds will normally cause the systolic pressure to increase about 20 mm. Hg.

(3) *Ophthalmoscopic examination* of the fundi of both eyes will give at once knowledge of the condition of the blood vessels, especially those of the nervous system. In the retina arteries and veins may be observed uncovered by any overlying tissue. They may be straight or tortuous, lined with the perivascular streaking of sclerosis or obliterated. There may be the flame-shaped retinal hemorrhages and the exudations so characteristic of advanced chronic nephritis.

(4) *Examination of a single fresh specimen* of urine should be made. This will include description of color, turbidity, reaction, specific gravity and the presence or absence of albumen and sugar. It frequently happens in cases of chronic nephritis especially of the so-called chronic interstitial nephritis, that albumen is absent from the urine while casts are present in greater or less numbers. For this reason it is essential that examination of the sediment after centrifugalization be made as a routine. Even where a renal damage is not suspected microscopic examination of the urine is indicated in patients giving a history of polyuria, especially of nocturia or showing signs of arteriosclerosis or hypertension and, again, when the urine examination shows a low specific gravity or the presence of sugar or albumen. Cases of nephritis in which albumen is present in fairly large quantities and which show an essentially negative sediment are in a fairly high percentage of histories associated with syphilis, and when such findings are noted a Wassermann reaction is indicated.

(5) *Mosenthal's special two-hour renal test* will be the first special test to be described because it is that test which shows the earliest and slightest degree of kidney damage in chronic nephritis. This test will show positive results at a time when the phenolsulphonephthalein test and the Ambard test will still be essentially negative. The performance of this test is indicated under the same conditions as noted for the examination of urinary sediment in the last paragraph. The finding of casts in the urine is also a direct indication for the test. The complete test consists, briefly, in placing the patient on a special diet in which the intake of salt, protein and water is known and controlled. Urine is collected in separate containers, at two-hour intervals throughout the day, the night output (9 p.m. to 7 a.m.) being collected as one specimen. The specimens which altogether would make one 24-hour collection are now examined separately as regards volume, specific gravity, salt content and total nitrogen content. As a rule the patient has been kept on a standard diet for two or three days preceding the day of the test. In this way the salt and protein intake are known to have been normal previously and there has been no shortage or too great abundance of either one, either of which conditions could tend to alter the results of the test.

In early chronic nephritis the kidney loses the ability to concentrate excreted substances. As a consequence one of the earliest urinary findings in

chronic nephritis is an increase in the fluid output with lowered specific gravity due to the lessened concentration of the solids. The subjective symptoms at this time are those of polyuria and nocturia. Whereas a normal kidney can rapidly relieve the blood of too great a concentration of solids, such as a high sodium chloride content, following a meal containing an excess of salt, by excreting it in high concentration, the damaged kidney has lost that power. When in the noon meal of the test diet an excess of salt is added (up to 6 gm.), the normal kidney responds as evidenced in the examination of the next one or two two-hour urine collections by secreting a urine of high salt content and high specific gravity. Long before the next meal the urine is back at its normal concentration and there remains no evidence of the excess burden that was recently put on it. The damaged kidney must take a much longer time to rid the body of the surplus and the resulting urinary picture shows only slight increase of sodium chloride concentration and of specific gravity, but a prolonged increase of urinary volume which may last even over the period of the next meal.

The normal kidney at night is able to concentrate the urine to such an extent that the amount voided after eight or more hours in bed is about the same volume as that voided at any one time during the day. The damaged kidney on the contrary excretes at night increased volume of a lower specific gravity. This accounts for the nocturia.

First in point of time, then, there develops fixation of volume and of specific gravity with increase in the night volume, next fixation of salt excretion with approximately the same amount excreted in every two-hour period and still later, fixation of total nitrogen excretion. The kidneys retain their ability to concentrate nitrogenous excretory products longer than they do the capability of concentrating sodium chloride. As the renal damage progresses the kidneys come to behave less and less like a functioning glandular organ and more and more like a mechanical filter.

The two-hour renal test as adapted to use in private practice may be outlined as follows:

(A) Direct the patient to eat on the two days preceding the collection of the urine as well as the day of the test ordinary meals such as are served in the average American household.

(B) On the day of the meal he is to take an excess of salt at the noon meal. He should distribute a level teaspoonful in his food in whatever manner his tastes may dictate.

(C) The patient's fluid intake should be that to which he has been accustomed in his daily life fairly evenly distributed over the day.

(D) At 7 A. M. of the day of the test he should void and discard the urine. Then every two hours, beginning at 9 o'clock, he should void and the individual collections should be kept separately in a cool place. The collection from 9 P. M. until 7 A. M. should be made in one container. As far as the patient concerned his part of the experiment is completed when he has voided at 7 A. M. into the night specimen.

(E) After all of the specimens have been collected volume and speci-



gravity of each should be noted. Determination of salt content and total nitrogen content require considerable special apparatus. Since variations from the normal in the manner of their excretion occur only after fixation of volume and of specific gravity has taken place, determination of the condition of the two latter is entirely sufficient for diagnostic purposes. Normally the specific gravity should at times be above 1018, particularly after meals. With the specific gravity constantly below 1018 there is present "fixation of specific gravity." There should be some definite variation of urinary volume among the two-hour specimens. The night specimen (including both the collections of 9 P. M. and 7 A. M.) should not measure over 400 c.c. volume. Its specific gravity should be 1018 or above.

(6) *Phenolsulphonephthalein Test*.—The technic of this test as originally described is capable of considerable simplification without disqualifying alteration of the end result. Two hours are required for the completion of the test. The apparatus necessary consists of one hypodermic syringe, a needle for intramuscular injection, ten or twelve tubes of practically constant diameter, a 1000 c.c. graduate, 40 per cent sodium hydroxide solution and the reagent phenolsulphonephthalein. This reagent may be obtained from several manufacturers put up in sterile 1 c.c. ampules. The contents of one ampule is to be injected.

The patient is directed to void. This urine is saved. One c.c. of the dye phenolsulphonephthalein is injected intramuscularly either into the deltoid muscle (care being taken not to injure the periosteum of the humerus) or into the gluteal muscles. The patient now drinks 200 c.c. (one glass full) of water. At the end of one hour he is to drink 200 c.c. more water and two hours from the time that the injection was made the patient again voids. This second urine specimen is to be examined to determine what per cent of the "phthalein" has been excreted during the period. Normally about 60 per cent should be present in the urine. A return of 40 per cent or less indicates a moderately advanced nephritis. The severity of the disease is greater as the amount of dye returned is less and less. An excretion of less than 10 per cent in the two-hour interval indicates severe renal damage and calls for a guarded prognosis. Usually at this stage it is a question of months or less, although exceptional cases, under careful treatment, have been known to live six or eight months with kidneys so diseased as to excrete practically none of the "phthalein" in the period.

In one condition other than nephritis the excretion of phenolsulphonephthalein may be reduced, namely, in chronic passive congestion of the kidneys such as develops in cardiac decompensation. Here the appearance of the dye in the urine may be delayed and unless this factor is taken into account the decrease in total two-hour output may be exaggerated.

An excretion of large amounts of the dye (80 per cent in two hours) indicates a hyperpermeable or better, hyperirritable, condition and is sometimes found in acute nephritis and in the early stages of chronic nephritis following the acute form. As chronic nephritis progresses the curve of "phthalein" excretion first rises and then falls and at one stage during the fall reaches 60 per

cent excretion. If one should happen to do the test only at this stage of the disease he would erroneously conclude that the renal function was normal. It is here that the two-hour renal test would provide the needed additional information. Not every case of acute nephritis is accompanied by this hypersecretion of dye. In some cases the excretion is very low and will be found to improve along with improvement in the disease itself. An excretion as high as 80 per cent in two hours will occur occasionally in normal individuals.

To determine the per cent of dye excreted the urine voided at the end of the two-hour period is placed in a 1000 c.c. graduate and measured. There should be present not less than 50 c.c. If there is less than this amount the results are untrustworthy since the subnormal excretion of water usually has an accompanying reduced excretion of the dye. The urine is next rendered strongly alkaline with concentrated sodium hydroxide solution. The white solution, or solution B, used in the determination of Fehling's test, may be substituted for the sodium hydroxide. Five or ten c.c. of the alkali are usually sufficient. It may be added until the resulting redness of the fluid ceases to increase in intensity. The urine is now diluted with water up to 1000 c.c. and thoroughly mixed. A specimen of this diluted urine is now taken and compared against a standard specimen in a graduated colorimeter and the reading directly obtained in per cent of dye. The colorimeters used are expensive and since the war began difficult to obtain. They are not essential. A satisfactory colorimeter consists of test tubes containing 10 per cent, 20 per cent, 30 per cent, etc., up to 90 per cent concentration of the dye. These are set up in a rack with just enough space between them for the insertion of a similar test tube containing the diluted alkaline urine. The color of this fluid is matched against the fluids in the series of tubes and it is not difficult to determine between which two tubes the urine tube should be placed. This colorimeter is accurate to within 5 per cent. For example, if the urine contains between 40 per cent and 50 per cent of the dye 45 per cent will be accurate within this limit. The colorimeter should be made up fresh at frequent intervals and readings are more easily made if the patient's own urine is used in the standard. This will insure there being about the same admixture of amber hue in both the standard and the urine to be tested, and it is for this reason that the urine passed at the beginning of the test is saved. To this urine when put in the liter graduate add exactly 1 c.c. of phenolsulphonephthalein, add alkali and make up to one liter. In the first tube put 1 c.c. of the diluted fluid and add 9 c.c. of water. This is the 10 per cent standard. Into the second pour 2 c.c. of the fluid and 8 c.c. of water, making the 20 per cent standard. Continue this up to the 90 per cent standard. Upon prolonged exposure to light the dye becomes less intense so for this reason it should be kept in the dark and the standard should be made fresh every time.

(7) *Ambard Test, McLean's Index of Urea Excretion and Blood Urea Nitrogen Content.*—The determination of these points requires both considerable experience and expensive apparatus. Here the amounts of urea nitrogen in the circulating blood and in the urine are determined, and at the same time

the rapidity and effectiveness of its excretion from the blood into the urine is expressed in terms of a coefficient or index of urea excretion. This corresponds quite accurately in nearly all cases to the per cent of phenolsulphonephthalein excretion. The latter is a much simpler test, has fewer sources of possible error and gives as much information. The particular advantage of the former is that we are determining the excretion of the actual substance in which we are interested, urea nitrogen, whereas in the other case we but determine the excretion of an inert dye and conclude that the excretion of urea and other waste products proceeds in a similar manner. The determination of blood urea nitrogen is important in cases of nephritis with impending uremia and should be made when possible but it is too technical and complicated to be successfully accomplished by any other than the trained laboratory man.

(8) *Therapeutic Test.*—One additional test should be described as being of value in enabling the physician to distinguish between the edema of acute nephritis and generalized edema of other origin. It occasionally happens that one is uncertain whether the anasarca in a certain case is the result of acute nephritis or cardiac decompensation from chronic myocarditis or other cardiac arrangement, or whether the patient presents a combination of both diseases. Cases of mediastinal tumor or even of cirrhosis of the liver will on rare occasions present a distribution of the edema much more suggestive of nephritis than of the actual disease present.

These patients are bedridden and will presumably have been placed on a low salt diet with very limited fluid intake. A very satisfactory diet to rid a patient of general edema is the Karrell diet which consists of 800 c.c. of milk during the twenty-four hours, given in four portions at four hour intervals. The patient receives no other food or liquid. If while the patient is on this diet he does not rapidly excrete the excess water in his tissues, the question as to whether or not this failure can be due to the presence of an acute nephritis may be settled by the administration of divided doses of theocine. This drug which like caffeine appears to act directly on the kidney as a powerful diuretic will as a rule cause a profuse diuresis in all the above diseases with the exception of acute nephritis and in this latter condition the fluid excretion may even decrease. Stimulation of the damaged renal parenchyma does not improve the kidney function. As a corollary it should be inserted that diuretics in the routine treatment of acute nephritis should be discredited. Before the drug is administered the patient should have been resting in bed and have been on a low fluid intake such as the Karrell diet for at least four or five days in order that an equilibrium of intake and output may have been established.

Christian recommends the giving of approximately five grain doses three times in one day at 6 A. M., 10 A. M., and 2 P. M. The resulting diuresis may persist for a period of two days or more. The drug should not be repeated for several days. There often appears to be a tendency towards slight depression of the excretory function for a day or two following this diuresis. Theocine, theocine sodium benzoate or the newer preparation, theophyllin, may be used in the performance of this test.

## DISCUSSION

By the use of the above described procedures one is now able to not only make the positive diagnosis of nephritis but also to ascertain to what stage the disease process has advanced.

The theocine test is of importance in acute nephritis. At this time salt excretion is greatly reduced. The phenolsulphonphthalein test may show a lowered excretion or an increased excretion through the hyperirritable renal cells. As the condition becomes chronic the "phthalein" excretion tends to fall as does the McLean index of urea excretion and there develops a fixation of volume and of specific gravity, followed by fixation of salt and total nitrogen excretion.

In the more marked stages of chronic nephritis we have the familiar picture with hypertension, arteriosclerosis and cardiac hypertrophy. The terminal stages bordering on uremia are evidenced by a "phthalein" excretion below 1 per cent, frequently albuminuric retinitis and a markedly increased blood total nonprotein nitrogen content. Whereas the normal is under 30 mg. per 100 c.c. of blood, in impending uremia it may rise to 60 or 100 mg. or higher.

The following table from Mosenthal and Lewis illustrates well the laboratory findings that are to be expected at the various stages of nephritis:

DEGREE OF IMPAIRMENT OF RENAL FUNCTION	PHENOLSUL- PHONEPH- THALEIN PER CENT	NONPRO- TEIN N <sub>2</sub> OF BLOOD MG. PER 100 C.C.	UREA N <sub>2</sub> OF THE BLOOD MG. PER 100 C.C.	TEST MEAL FOR RENAL FUNCTION					
				NIGHT URINE		VARIATIONS IN SP. GR. WHEN HIGHEST IS			
				C.C.	SP. GR.	18	17-15	14-13	12
Normal 0	60+	30-	15-	400-	18+	9+			
Slight +	59-40	31-45	16-27	401-600	16-17	8-5	6+		
Moderate ++	39-25	46-65	28-44	601+	15-	4-	5-4	6+	
Marked +++	24-11	66-90	45-64	...	...	3-	5-4	6+	
Maximal ++++	10-0	91+	65+	...	...	...	3-	5-	6+

Hypertension and arteriosclerosis do not invariably accompany chronic nephritis. Any one of these conditions or any combination of two of them may be found in one patient. High blood pressure, unaccompanied by marked nephritis, so-called "essential hypertension," usually shows moderate renal damage as indicated by positive findings in the two-hour renal test. The other special tests may remain essentially negative. In time there is a decrease in renal efficiency but death is more liable to be from apoplectic phenomena than from uremia.

The classification of nephritis by the above described laboratory methods is functional rather than anatomical. No mention has been made of chronic interstitial nephritis as contrasted with the chronic parenchymatous variety. We are not so much interested in whether it is the glomeruli or the tubules that are damaged as we are in whether the kidneys may satisfactorily care for the body waste. Recent investigations have shown that if one contents himself with the finding of "chronic nephritis" he will be correct far oftener than if he endeavors to elaborate upon this diagnosis. At a time when pathologists are still disputing among themselves concerning the character of renal lesions found at autopsy it is well for the internist not to endeavor from examination of the living subject to describe the location of the lesion in the kidneys themselves.



### THREE CASES OF PARIETAL AORTIC THROMBOSIS\*

BY PAUL G. WOOLLEY, M.D., CINCINNATI, OHIO.

THE following cases are of interest because the symptoms were associated with, and perhaps largely the results of, partial, i. e., incomplete aortic thrombosis. Two were patients on the neurologic service; one was on the medical service. All were evidently luetic. In one, the central nervous symptoms were acute; in the others, chronic. One patient was an old man. Two were young.

#### CASE I

G. B., Hospital No. B-1578, a white man aged 35, was admitted to the Cincinnati General Hospital on March 2, 1917, in a somewhat maniacal condition. He would not respond to questions and talked irrelevantly and foolishly. The following history was obtained from his physician:

An uncle of the patient had had an acute maniacal attack, similar to that of this nephew, at the age of 40. During this attack he was sent to an asylum where he died later. A daughter of this uncle—a cousin of the patient—also developed acute mania, at the age of 35, and was committed to an asylum where she now is. A father of this uncle was also confined in an asylum. The history of his condition is not known. For at least two weeks prior to the present outbreak, the patient had been apparently perfectly normal mentally. At the time of the attack, which took place in a store, the patient's pupils were not fixed, and so far as the doctor knows they have never been fixed before.

The following history was obtained from a friend: On Tuesday, Feb. 28, 1917, the patient was working as usual in a grocery store when suddenly, it was noticed, he could not speak. He stepped away from the counter in a dazed way and proceeded to leave the store. His employer called to him but he continued his course. The employer then ran after him and with great difficulty brought him back fighting and incoherently mumbling. Upon reaching the store the patient had four convulsions in rapid succession. These began in both arms, and involved the entire body. The mouth opened and closed. The tongue could have been bitten if a piece of wood had not been inserted between the teeth. There was no cry. The convulsions seemed to be bilateral. Each convulsion lasted from 1 to 2 minutes, and after they had disappeared the patient remained stuporous for some time. On Wednesday the condition seemed to be unchanged. On Thursday he began to talk at random, foolishly and incoherently. On Friday he was brought to the hospital. The patient has been married a year. His wife is five months pregnant. He is not a drinker. An uncle thinks that he uses no alcohol.

*Present State.*—The patient is a poorly nourished, poorly developed white man aged about 35 years who refuses to cooperate in his examination. He can not be drawn easily into conversation. His memory can not be estimated for he refuses to respond to questions. When a question is put to him he grins foolishly, stutters and usually says something containing the letters p and t, and the figures 2, 1, and 10. The sentences are absolutely meaningless. He seems to see and hear normally but this is not secure. The right pupil is slightly larger than the left. Both react slightly to strong light. The reaction of accommodation could not be determined because of lack of cooperation. The patient opens his mouth mesially. He does not flinch when the face is forcibly pricked with a pin. There is no facial asymmetry and the facial movements seem normal. There is decided stumbling speech, stuttering, and syllable skipping. The pulse is normal. The patient refuses to protrude the tongue which lies quietly in the median line in the mouth. There is no evident sensory disturbance for pain. The patient does not flinch when pricked with a pin. There is no corporeal asymmetry and no evident loss of power. There are no conjunctures, tremors or spasms. The knee jerks, arm jerks, and periosteal reflexes are ex-

\*From the Mary M. Emery Department of Pathology of the University of Cincinnati, and the Pathologic Institute of the Cincinnati General Hospital.

aggerated. The abdominal and cremasteric reflexes are absent. There is no clonus, and no Babinsky. The gait is normal.

During the night of March 4th, the patient became very noisy. No tremor developed. The temperature rose suddenly to 103° and the pulse to 112. The patient was forcibly restrained and fought all day long at the shackles and restraining sheet. At four o'clock p.m., he was given Hogan's solution intravenously. This quieted him for about 2 hours. He then became violent again. On the following day the temperature was 104, the pulse 116. All day he was wildly delirious and fighting his restraint. He was tremorous, and muttered continually to himself. The pupils were wide, equal and did not respond to light. During the following day the condition remained the same—noisy, muttering, delirious with high temperature. There was no rigidity or retraction of the neck. The next day he was pulseless but constantly delirious and muttering. The pupils remained dilated and fixed.

Death occurred at 10 A.M., on March 9, 1917.

The urine on March 5th was milky, yellow, cloudy and acid. It contained a trace of albumen but no sugar or casts.

*Clinical Diagnosis.*—Acute paresis, acute diffuse nephritis.



Fig. 1.—Case B. 1578. Note the pedunculated thrombus attached to the aortic wall.

#### AUTOPSY PROTOCOL

The body was that of a fairly well built, rather slender man of not more than 30 years of age. There was a very slight edema of the ankles. Rigor mortis was not present. Postmortem lividity was faint. Over the wrists and elbows were superficial excoriations and over the sacrum, there were recent, shallow, somewhat inflamed, slightly ulcerated excoriations. The pupils were dilated, the left slightly more than the right. The left foot, extending about 1 or 2 inches above the ankle, was cyanotic. The finger nails were pale. Over the thorax and in the hypochondriac region on the left side were a number of pale greenish bruises.

The brain weighed 1410 grams, was markedly congested and tremendously edematous, dripping fluid. The dura was not abnormally adherent. The pia was thickened and opaque, particularly over the frontal aspect of the brain. There was a definite atrophy of the frontal convolutions with flattening and widening of the sulci. Upon palpation the brain seemed to be more sclerotic than usual. This was more apparent on the right side. The vessels were not sclerotic. The brain was preserved in formalin for further study. In the spinal canal there was a considerable increase of perfectly clear limpid fluid. The subcutaneous tissues were well developed. The muscles were fairly well developed, dark in color and dry. The lower margin of the liver was 4.5 cm. below the tip of the ensiform cartilage and at the costal margin in the right mammillary line. The urinary bladder was distended

with urine. The intestines were small and congested, but there was no evidence of inflammatory change. The appendix was *in situ*. The omentum was coiled up along the transverse colon.

When the sternum was removed, the lungs collapsed partially. In the left pleural cavity was one small band of adhesions posteriorly at the upper part of the lower lobe. The right lung was completely free. There were no adhesions between the lobes. There was a very small amount of anthracosis. Both lungs appeared, grossly, completely healthy. In the left main bronchus was a small amount of thick greenish pus. There was a small amount of thymus remains. The mesentery was well supplied with fat and slightly edematous.

The right kidney was soft, pale, succulent, its capsule came off with ease, leaving a perfectly smooth surface. Here and there upon the surface were rectangular areas of pale yellowish material surrounded by definite zones of congestion, which resembled anemic infarcts. The cortices in each kidney were of less than normal thickness. The whole organ was rather firm, the friability decreased, the line of demarkation between cortex and medulla rather faint. There was an increased amount of perirenal fat and the pelves were healthy. The left kidney resembled the right.

The spleen was small and rather flabby, except at one area where there was a large infarct, mottled in character, measuring 4 cm. at its greatest depth and 3.5 cm. wide.

The heart was small, somewhat dilated and flabby. On the right side it was contracted, and firm on the left. There were no auricular thrombi in the right auricle. There were no pulmonary or tricuspid lesions. There were no thrombi in the left auricle or auricular appendage. The mitral orifice permitted the passage of two fingers. The mitral leaflets were somewhat edematous but otherwise not abnormal. The aortic valves were perfectly healthy apparently except for a few adhesions at their points of connection, but just 1 cm. above the junction between the right and posterior aortic cusps, in a hyaline hyperplastic area which seemed to be of luetic origin, was a small ulcerated surface covered with a small amount of thrombus. Lying free in the aorta just above this was a piece of thrombus that measured 2.5x1.5x1 cm., which was firm, pale and evidently cavitated. In the transverse arch just external to the mouth of the subclavian was another area similar in every respect to that above the aortic valve to which was attached a long pedunculated, white, fibrinous thrombus. There was comparatively little change in the aorta aside from some indistinct puckering which was most marked in the transverse arch and a considerable amount of fatty degeneration which extended throughout the aorta.

The left lung was apparently perfectly healthy except for one small patch of fibrosis and calcification immediately beneath the adhesions. The right apex was scarred. There were also a few old adhesions between the middle and upper and lower lobes. Just at the apex of the lower lobe there were some patches of congestion with some evident consolidation beneath them. On the pleura there was no exudate. In the lower part of the lower lobe there were several other similar areas and posteriorly there were a few more that resembled these upper ones. Over none of these was there any exudate. In some of the bronchi there were small amounts of yellowish mucous purulent material. These areas of congestion in this lobe were apparently areas of inflammatory edema. There was a larger posterior one which seemed to be a very recent infarct for in the vessel supplying it a thrombus was found.

The liver was almost completely healthy externally except for the presence of a few nodular linear markings. On section, the organ showed some patchy fatty change and some moderate congestion. The gall bladder was filled with a brownish thick mucoid bile. There were no calculi present. Except for some patchy ecchymoses in the ileum there seemed to be no lesions in the intestines.

The stomach was small and contained a small amount of bile-stained fluid. There was nothing abnormal in the stomach or duodenum. The pancreas was healthy.

**Anatomic Diagnosis.**—Luetic mesaortitis; aortic mural thrombosis; renal, pulmonary, splenic infarcts; chronic diffuse nephritis; obsolescent pulmonary tuberculosis; purulent bronchitis; edema of the brain.

#### REMARKS

It is our opinion that the acute nervous symptoms in this case were immediately due to numerous small cerebral emboli which reached the brain by way

of the carotids from the thrombus in the aorta. At least, it were better to say, we feel that these symptoms may have been due to such a phenomenon. There was no macroscopic evidence of cerebral embolism. How much influence the abnormal mental background had in the picture—in the production of a susceptibility to trauma—can be only guessed. There can be no doubt about the background. There is also anatomic evidence that the brain as a whole was not a normal one. Sections of the cerebral cortex showed perivascular infiltrations and edema, and round-cell infiltrations of the pia,—evidences of paresis. Sections of the cord showed no suggestive changes. (W. E. Kiely.)

#### CASE II

C. S., Hospital No. B-1043, a white man 61 years old, was admitted to the Cincinnati General Hospital on Feb. 12, 1917, in a semiconscious condition. He attempted to answer questions but was unintelligible. When asked to protrude the tongue he responded. The

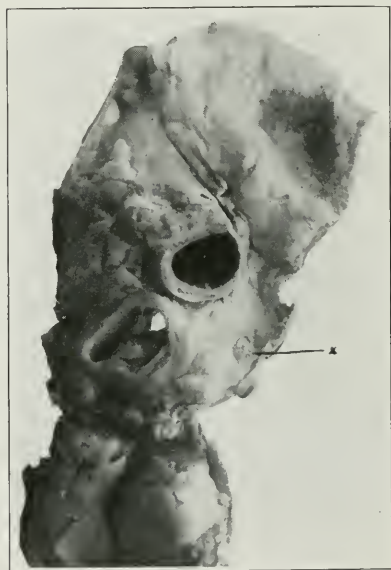


Fig. 2.—Case B, 1043. The aortic arch (x), from the outside showing the dilated innominate artery with its parietal thrombus propagated for the aorta, and to the right of this vessel, the completely blocked carotid. The wide opening above the innominate is the trachea.

tongue deviated to the right. He was unable to hold up the right arm or move the right leg. He was able to move the left arm and leg. The pupils reacted to light, and the left pupil seemed smaller. The arm jerks were about equal, though possibly the left was greater. The abdominal and cremasteric reflexes were present on the left; absent on the right. The knee jerk was diminished on the right; exaggerated on the left. There was no ankle clonus. On the right the Babinski sign was present; on the left it was absent. When the sole of the right foot is tickled the patient draws the leg up weakly. The heart sounds were clear and there were no murmurs. The patient appeared drowsy and yawned frequently.

From a niece it was learned that the patient had not been working for about 3 years but has seemed well until the time of the present attack which began while he was riding in a street car. A neighbor who was in the same car helped him off, and with difficulty brought him home. No accurate description of the attack was obtained although it was determined that there were no convulsions.

On Feb. 15, a slight ankle clonus appeared on the right, though the general condition of the patient remained about the same.

On Feb. 25, the patient was slowly sinking, and on March 11, he died.



During the hospital period, the patient's pulse ran from 88 to 134; the temperature from 97° to 101°; and the respirations from 20 to 24.

*Clinical Diagnosis.*—Cerebral hemorrhage; lues.

#### AUTOPSY PROTOCOL

The body was that of a well-built, fairly well-nourished man of about 55 years of age; rigor mortis and lividity were present. There was no edema of the ankles. The finger nails were somewhat cyanotic. The eyelids were glued together with a purulent secretion. The pupils were equal, neither dilated nor contracted. Just external to the right eye was a focal dermatitis with some suppuration. On either side of the neck beneath the ears, there were areas of cutaneous hyperplasia, larger on the left, which in some way resembled nevi, and were covered with hair. The edges were rather sharp, however, although not regular. The skin over them was thickened; and, extending from the posterior margin on either side, there were excoriated areas which measured not over  $\frac{1}{2}$  cm. in diameter, in which there were distinct evidences of inflammation. These smaller patches resembled the patches on the right of the right eye. The peripheral lymph glands were not appreciably enlarged. The teeth were in rather bad condition; the gums were quite pyorrheic. There was one old crown and several fillings in the upper teeth. The subcutaneous tissues were very well developed; the muscles were only fairly well developed but of good color. The peritoneum was thickly filled with fat and was partly coiled up above the transverse colon and stomach. The appendix was present. There was no obvious lesion in the abdominal cavity except a few old adhesions between the gall bladder and hepatic flexure of the colon and other numerous old adhesions about the spleen. The lower border of the liver was 5.5 cm. below the ensiform and 3 cm. above the costal margin in the right mammillary line.

The brain was exceedingly edematous and the veins were all deeply congested. The brain contained so much fluid that it appeared gelatinous. Externally there was no evidence of any arteriosclerosis and there was no evidence of superficial inflammation. On section of the brain it was found that the ventricles were slightly dilated and throughout the substance of the brain on both sides the smaller vessels were congested. On the left side it appeared that almost the whole thalamic region and some of the surrounding white matter was generally destroyed. The degenerated softened tissue was for the most part almost white, although in some areas there was a tinge of yellow. The appearances suggested that there was a certain amount of suppuration present.

When the sternum was removed, the lungs did not collapse. In the left pleural cavity was one group of old lateral adhesions over the lower lobe and some old diaphragmatic adhesions. In the right pleural cavity there was an obliteration of about half the cavity. There was no increase of pericardial fluid. The mesentery was very well supplied with fat. The spleen was completely buried in old fibrous adhesions so that it was firmly adherent to the stomach, liver and diaphragm. There was a large increase of perirenal fat. The adrenals were cavitated.

The right kidney was small, pale, flabby, and decidedly edematous. The capsule removed with only slight difficulty and left a fairly smooth surface. The capsule, however, separated, showing a very finely granular surface. At one place was a scarred area that resembled an infarct scar. The capsule was somewhat thin but gray. The glomeruli were not visible. The line of demarkation between cortex and medulla was faint. The stellate veins were somewhat injected. The pelvis was healthy. The left kidney resembled the right in all respects except for the presence of the scar. The blood vessels of the kidney were somewhat patulous.

The heart was large and flabby and, particularly on the right, dilated. The coronary vessels were not tortuous and not evidently sclerotic. In the right auricle was a large mural clot. There was no lesion of the tricuspid or pulmonary valves. There were no thrombi in the left auricle. On the mitral valve was an adherent clot and the edges of the mitral leaflets were somewhat thickened. The clot upon the mitral valve was evidently one of not very long standing (agonal) and at the point of attachment on the valve there was no lesion. The aortic leaflets were healthy except that at the point of attachments there was a certain amount of sclerosis. The aorta itself was tremendously and diffusely changed by an atherosclerotic process which had led to subintimal hyperplasia, thickening, wrinkling, and to the presence of atheromatous ulcers, and abscesses together with calcification. Just at the apex of the arch there was a large clot that was adherent to a ulcerated plaque just at the opening of the innominate artery which was considerably

dilated. This clot extended from the opening of the innominate to the origin of the carotid which was completely blocked for some distance by a mixed lamellated thrombus. The aorta, throughout its thoracic portion, was considerably dilated, and just in the region of the innominate the dilatation was more distinct and acquired a saccular form. It was from the apex of this saccular secondary aneurism that the dilated innominate originated. The myocardium was flabby and for the most part pale, but particularly in the papillary muscles, there were zones of deep congestion alternating with areas that were paler, somewhat yellow, indicating some deposition of fat. The mural endocardium was almost generally thickened. The coronary openings were patent.

The left lung was crepitant throughout, contained no areas of consolidation and was merely mildly congested. The right lung presented the same appearances. The spleen thickly covered with the remains of old adhesions, was exceedingly soft. The malpighian follicles were visible as small points. The substance had no increase in connective tissue was almost diffuent and of a brownish-red color. The liver was large and flabby. The surface had a couple of grooves across the dome on the right. The whole organ was flabby. Section of the parenchyma showed a mottling due to some moderate degree of passive congestion evidently, with slight central lobular atrophy. The whole organ was edematous. In the left lobe the evidences of passive congestion were more brilliant. Aside from the moderate increase in mucus, the stomach and duodenum showed nothing unusual. The pancreas was slightly congested and, particularly in the tail, there seemed to be some areas of parenchymatous hypertrophy, in evidence of which were islands of very pale fat tissue. There was very little splanchnic arteriosclerosis.

*Anatomic Diagnosis.*—Luet's mesaortitis; dilatation of the aortic arch; aneurism of the aortic arch including the innominate artery; aortic mural thrombosis; thrombosis of the left carotid artery; cerebral embolism; edema of the brain and meninges; chronic diffuse nephritis; myocardial degeneration; hypertrophy and dilatation of the heart; pyorrhea alveolaris.

#### REMARKS

In this case, there can be no doubt of the immediate cause of the symptoms. They were due to cerebral embolism. If, in the former case there were very small emboli, in this case they were large and produced focal lesions that were easily discovered.

#### CASE III

E. M., Hospital No. A-3062, a negro 23 years old, was brought into the receiving ward of the Cincinnati General Hospital, on May 1, 1916.

No history could be obtained. All that could be learned was that he had come from a neighboring town two days before. On admission, his temperature was 102°; pulse 110; respirations 40.

The patient is a powerfully developed man. He is lying on his back and can not be roused. His respirations are deep and irregular. The pupils are pinpoint in size and equal. They fail to respond to stimuli. On the right malar prominence there is a superficial abrasion but no swelling. There is no swelling of the face. There is no stiffness of the neck. The left angle of the mouth is pulled slightly outward and there is an occasional twitching of the muscles in this region. The neck is negative. The thorax is well formed and symmetrical. Expansion is equal but rather small. The lungs are clear throughout on auscultation and percussion.

The point of maximum cardiac impulse is 9.5 cm. to the left of the midsternal line in the fifth interspace. The impulse is rather diffuse and fairly strong. The relative cardiac dullness extends 10.5 cm. to the left of the midsternal line, and 6.5 cm. to the right. There is no retrosternal dullness. Both sounds are strong and forcible at the apex, but not so strong at the base. On auscultation there is a faint systolic murmur heard at the apex, but louder at the left border of the sternum in the third and fourth interspaces. It is not transmitted beyond this region. The pulse is regular in force and rhythm. The blood pressure is 160 systolic, 94 diastolic; pulse pressure 66.

The abdomen is just below the level of the ribs and is symmetrical. There is no rigidity or tenderness. The liver and spleen are not enlarged.

The abdominal and cremasteric reflexes are absent. Knee jerks are not obtained. Plantar stimulation elicits a very slight plantar flexion of the great toe. Oppenheimer's reflex is absent. There is no clonus. There is no spasticity of the upper or lower extremities.

The urine obtained in the receiving ward contained a considerable amount of albumin. A specimen obtained by catheterization the following morning contained a small amount of albumin, no sugar, acetone, or diacetic acid, and coarsely granular casts.

Blood examination gave 4,500,000 red cells; 8,600 leucocytes, and 80 per cent hemoglobin. A differential count gave 23 per cent lymphocytes, 12 per cent mononuclears, 4 per cent transitionals, 61 per cent neutrophils, no eosinophils, or mast cells. The urea content of the blood was 100 mg. The hydrogen-ion concentration was 7.65.

Shortly after admission 350 c.c. of blood were withdrawn at the elbow, after which the systolic pressure dropped from 160 to 138, without change in the diastolic. An intravenous injection of a liter of Fischer's solution was given after venesection and following this the blood pressure rose to 180 systolic, and 100 diastolic.

Spinal puncture was done and about 50 c.c. of fluid was removed, apparently under no increased pressure. The fluid was blood-tinged. Immediately after this the patient roused sufficiently to give his name and age. There was no change in the pupillary reaction, and he soon relapsed into coma. The following day spinal puncture was repeated, and the fluid obtained was not under any increased pressure. There was a small amount of blood in each of the four tubes in which the fluid was collected. A spinal Wassermann was negative; a blood Wassermann was strongly positive.

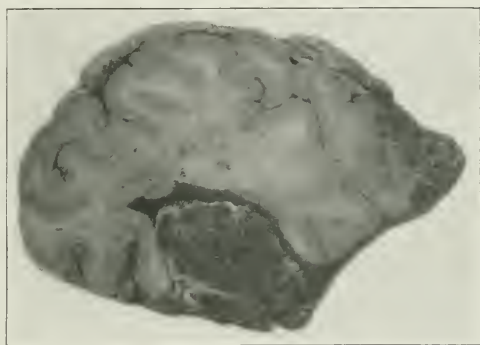


Fig. 3.—Case A. 3062. Central infarction (embolism).

The patient's condition remained unchanged throughout the day. At 6:30 P.M. the temperature rose to 106.6°; the respirations became labored; the pulse was fair. The pupils became extremely dilated and fixed. Ophthalmoscopic examination showed nothing unusual. The blood pressure was 130 systolic, 100 diastolic.

The patient died at 7 P.M., on May 2, 1917.

*Clinical Summary.*—A colored man, 23 years old, was admitted in coma. He was well developed, the pupils were equal and pinpoint. The reflexes were abolished. The heart was dilated and a systolic murmur was heard at the apex, transmitted toward the base, with its maximum intensity in the 3rd and 4th interspaces near the sternum. The blood pressure on admission was 160 systolic and 94 diastolic. The eyegrounds were negative on ophthalmoscopic examination. The urine contained some albumin and coarsely granular casts. The blood urea was 103 milligrams. The spinal fluid contained red blood cells.

*Clinical Diagnosis.*—Pachymeningitis interna hemorrhagica; chronic nephritis; chronic myocarditis; hypertrophy and dilatation of the heart.

#### DISCUSSION (R. S. M.)

The lack of a history is unfortunate. There could be found no evidence of trouble sufficient to cause the present condition and no evidence of poisoning could be found. The urinary findings were against acute nephritis, although the secretion of urine during the last day of the patient's illness was much diminished. The increase of the blood



urea points to renal insufficiency. The blood pressure was moderately elevated. The spinal fluid on two occasions contained blood which apparently did not come from the puncture wound, as it was uniform in all parts of each specimen. There were no focal nervous symptoms. The absence of these, together with the presence of blood in the spinal fluid and the moderate elevation of blood pressure led us to suspect pachymeningitis hemorrhagica interna. In a process as acute as this, the absence of focal symptoms, if this diagnosis were correct, would be somewhat puzzling. Uremia seemed probable from the increase of urea, the scanty secretion of urine and the elevation of blood pressure. The absence of changes in the eyegrounds, however, is noteworthy.

#### AUTOPSY PROTOCOL.

The body was that of an exceedingly well-built, well-nourished, colored man, probably 35 years of age. Rigor mortis was present, posterior lividity was also present, but not brilliant. Over the right malar region was an excoriation. The pupils were equal. The teeth were in fairly good condition, but all of them showed atrophy of the crowns. There was no pyorrhea. The finger nails were pale. In the bend of both arms were surgical wounds closed with silkworm-gut sutures. The subcutaneous fat was exceedingly well developed. The muscles were exceedingly well developed, of good color and dry. The omentum formed an apron over the anterior surface of the intestines and was well supplied with fat. The small intestine was for the most part contracted and the large intestine was dilated, chiefly with gas. The appendix was *in situ*, and apparently healthy. There was no abnormality of appearance or arrangement of the intestines. The lower border of the liver lay 5 cm. below the ensiform. There were many old veil-like adhesions between the anterior surface of the liver and diaphragm. When the sternum was removed, the lungs did not collapse. In the left pleural cavity there was no increase of fluid and no adhesions except between the surface of the lower lobe and diaphragm. There were no adhesions and no increase in fluid in the right pleural cavity. There was no abnormal increase in fluid in the pericardial cavity. The bronchial lymph glands contained old obsolescent tubercles.

The heart was excessively increased in size, the right side somewhat dilated and evidently slightly hypertrophic. The tricuspid and pulmonary valves showed very little change. The mitral seemed to be approximately perfectly healthy. The aortic leaflets showed a few adhesions at their points of insertion and the right one was fenestrated at its margin. Beginning above the aortic valves, there was very distinct syphilitic aortitis and about half way between the valves and the origins of the great vessels of the neck were several mural vegetations, one of them being 3.5 cm. in length by 1 cm. in width, the others being smaller. Also, in the thoracic aorta, just above the diaphragm there was another mural thrombus which measured 2 by 1.5 cm. in width. All of these were about 0.5 cm. thick.

The liver was of fair size, the capsule was irregularly thickened and upon it were numerous scars of old adhesions. The whole external surface was pale. On cross section, the parenchyma appeared to be cloudy, with here and there rather discrete areas of fatty degeneration. There was evidence of a certain degree of passive congestion, not however, distinctly chronic. The gall bladder was filled with a yellowish-brown, rather thick, mucoid bile and contained no calculi.

The right lung was somewhat voluminous and rather bluish-purple in color, and crepitant throughout except for a single area 1.5 cm. in diameter, just beneath the middle of the lateral surface of the lower lobe. There was no sign of pleuritis. On section the area of consolidation appeared to be an obsolescent fibroid tubercle with caseous center. The lung tissue was saturated with fluid and exceedingly congested. The left lung resembled the right. There were no areas of consolidation to be felt. The whole lung seemed to be somewhat more boggy than the right. On section, the same condition of intense congestion and edema was present. From some of the smaller bronchi, yellowish drops of pus were expressed and upon the cut surface of the lower lobe, one could feel and see very small areas of consolidation not over 1 or 2 mm. in diameter, which had an indistinct yellowish appearance, and pressure upon them forced out a creamy, yellowish pus. There were large numbers of these areas of consolidation throughout the lower lobe and some still smaller ones in the upper lobe. All of the finer bronchi contained pus. The bronchi were all intensely congested but evidently only in the smaller ones was there any purulent exudate.

The spleen was small and the surface was scarred with the tags of old adhesions. On cross section, the pulp was firm, congested and the malpighian bodies were easily



visible as discrete points. There were the healed remains of old tubercles. There was no evidence of increased fibrous tissue in the organ. The pancreas was exceedingly soft and evidently was undergoing autolysis. The stomach and intestines showed very well marked postmortem changes. In addition to this, the stomach showed some morocco-leather appearance. The duodenum showed nothing unusual. There was no evidence of passive congestion. There was nothing unusual in the intestinal tract. The mesentery was quite fatty. The mesenteric lymph glands were not evidently enlarged. All of the abdominal organs, superficially at least, were exceedingly dry. The spleen was surrounded by old adhesions, some of which were between it and the stomach. Neither adrenal showed anything macroscopically unusual.

Over the lower pole of the right kidney, the fatty capsule was infiltrated and more than usually adherent. The capsule removed with ease leaving a smooth surface which, as a rule, was pale, but was irregularly mottled with areas of hemorrhage, so that it had, particularly in the lower half, an appearance of marble. On section, it appeared that except for about a fourth of the parenchyma, the kidney was the seat of multiple infarctions. The left kidney was exceedingly large, like the right, and on section showed practically the same series of changes. It seemed possible, from the appearances in the kidney, that some of the infarcts were older than others, and at each inferior pole the process seemed to be most recent. For the most part, these infarcts seemed to be white; the most recent ones, however, were red.

"The dura was not thickened and showed no unusual changes. The pia showed an exceeding grade of congestion with, however, very little edema. The brain was rather dry. In the superior longitudinal sinus, just over the occipital lobes, was a definite, adherent, gray, friable thrombus. The convolutions were flattened and there was no accumulation of fluid between them. There was evidently no arteriosclerosis. Particularly the internal aspects and, to a certain extent, the external, ventricular, and inferior aspects of the occipital lobes were intensely and generally congested. All of the brain substance in these areas was evidently saturated with blood pigment and the areas that showed these hemorrhagic changes seemed to be softer than the rest of the brain. The whole appearance suggested red infarction. Examination of the brain after it had been hardened, revealed a large infarct on the inferior surfaces of the occipital lobes extending up through the cortex and about 1 cm. into the white matter. The left infarct passed up and forward above the cortex to a point just above the left cerebral peduncle; the right infarct passed up through the oliva and ended just beneath the corpus callosum at a point just above the middle of the optic chiasm." (Note by Chas. E. Kiely.)

*Anatomic Diagnosis.*—Aortic mural thrombi; cerebral and renal infarcts; thrombosis of the superior longitudinal sinus; dilatation of the right heart; luetic mes-aortitis; acute lobular pneumonia (bronchiolitis); healed obsolescent pulmonary and splenic tuberculosis; perihepatic and perisplenic adhesions.

#### REMARKS

This case is unusual in several respects. In the first place, thrombosis of the aorta is not a common occurrence. When it does occur, it is not infrequently followed by cerebral embolism, but not to the extent shown here. Complete infarction (red softening) of both occipital lobes is exceedingly unusual.

#### SUMMARY

Three cases are reported in which aortic thrombosis occurred at the seats of luetic lesions. From these thrombi, emboli were carried to various portions of the body. In one case the symptoms were acute; in one they were chronic and referred to paralytic disturbances; in one they were probably acute and paralytic. The varying distributions of the emboli from thrombi in essentially the same place in the aorta is interesting.

# LABORATORY METHODS

---

## TABLES FOR USE IN BLOOD ANALYSIS\*

BY FLORENCE HULTON-FRANKEL, PH.D., NEW YORK CITY

THE amount of calculation necessary in the laboratory of a large hospital where routine analyses are done, is considerable, and any device which minimizes this and introduces a greater degree of safety, from the standpoint of personal error, seems advisable. The following tables were compiled for use in this laboratory, but in view of the requests, from visitors from other laboratories, for copies of the tables it seemed that they might prove more generally useful if published.

Nonprotein nitrogen is determined in blood according to the method of Greenwald.<sup>1</sup> Five c.c. of blood are diluted in a volumetric flask to 50 c.c. with 5 per cent trichloroacetic acid and allowed to stand about one-half hour and then centrifuged and filtered. Twenty c.c. of this filtrate are digested with 4 c.c. of a digestion mixture consisting of potassium and copper sulphate and sulphuric acid, till colorless and then 10 c.c. of concentrated alkali is added and the ammonia formed is distilled into 15 c.c. of 0.01 N acid containing 3 drops of methyl red. The condenser is carefully washed and the washings run into the receiver. One drop of methylene blue is added to the distillate which gives a purple color, and the excess of acid is titrated with 0.01 N alkali. This indicator is very sensitive, one drop of 0.01 N alkali at the end point changing the color from purple to a vivid grass-green. The burette containing the alkali is read and the results read directly in milligrams of nonprotein nitrogen, from the table. This eliminates the necessity for subtraction, thus avoiding another possibility of error and saving considerable time in the course of a day.

The figures for the majority of cases will fall within the limits of those in Table I. The figures in the left-hand column represent whole cubic centimeters of 0.01 N alkali used in the titration. The figures in the headings across the page represent tenths of a cubic centimeter; i. e., if the burette reads 4.5 c.c., look down the first column to the left until 4 is reached, then across the page on the same line as 4 until the figure immediately below 0.5 is reached giving a reading of 73.5 mg. of nonprotein nitrogen per 100 c.c. of blood.

In ordinary routine analyses, closer readings than this are not necessary however in more accurate work when necessary, where the burette is graduated in 0.1 of a cubic centimeter, the intervening figures can be easily interpolated. A few cases, as in uremia, will show figures higher than those recorded in Table I. In this event the same amount of filtrate is distilled into 10 c.c. of 0.05 N acid and titrated with 0.05 N alkali and the results read in the same way from Table II.

\*From the Harriman Research Laboratory, Roosevelt Hospital, New York City.

TABLE I—NONPROTEIN NITROGEN

Take 20 c.c. filtrate for micro Kjeldahl, digest and distill into 15 c.c.  $\frac{N}{100}$  acid.  
Figures give mg. N. per 100 c.c. blood.

ALKALI USED FOR TITRATION										
c.c.	.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0	105	104.3	103.6	102.9	102.2	101.5	100.8	100.1	99.4	98.7
1	98.0	97.3	96.6	95.9	95.2	94.5	93.8	93.1	92.4	91.7
2	91.0	90.3	89.6	88.9	88.2	87.5	86.8	86.1	85.4	84.7
3	84.0	83.3	82.6	81.9	81.2	80.5	79.8	79.1	78.4	77.7
4	77.0	76.3	75.6	74.9	74.2	73.5	72.8	72.1	71.4	70.7
5	70.0	69.3	68.6	67.9	67.2	66.5	65.8	65.1	64.4	63.7
6	63.0	62.3	61.6	60.9	60.2	59.5	58.8	58.1	57.4	56.7
7	56.0	55.3	54.6	53.9	53.2	52.5	51.8	51.1	50.4	49.7
8	49.0	48.3	47.6	46.9	46.2	45.5	44.8	44.1	43.4	42.7
9	42.0	41.3	40.6	39.9	39.2	38.5	37.8	37.1	36.4	35.7
10	35.0	34.3	33.6	32.9	32.2	31.5	30.8	30.1	29.4	28.7
11	28.0	27.3	26.6	25.9	25.2	24.5	23.8	23.1	22.4	21.7
12	21.0	20.3	19.6	18.9	18.2	17.5	16.8	16.1	15.4	14.7

TABLE II—NONPROTEIN NITROGEN

Take 20 c.c. filtrate for micro Kjeldahl, digest and distill into 10 c.c.  $\frac{N}{20}$  acid.  
Figures give mg. N per 100 c.c. blood.

ALKALI USED FOR TITRATION										
c.c.	.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0	350	346.5	343	339.5	336	332.5	329	325.5	322	318.5
1	315	311.5	308	304.5	301	297.5	294	290.5	287	283.5
2	280	276.5	273	269.5	266	262.5	259	255.5	251	248.5
3	245	241.5	238	234.5	231	227.5	224	220.5	217	213.5
4	210	206.5	203	199.5	196	192.5	189	185.5	182	177.5
5	175	171.5	168	164.5	161	157.5	154	150.5	147	143.5
6	140	136.5	133	129.5	126	122.5	119	115.5	112	108.5

Creatinine and sugar are determined according to the Myers and Bailey<sup>2</sup> modification of the Benedict method—5 c.c. of blood are laked with 20 c.c. of distilled water and saturated with recrystallized picric acid. The saturation is accomplished by shaking the blood with the picric acid, in small stoppered flasks. The whole is centrifuged and filtered and 3 c.c. of the filtrate are used for sugar and the rest for creatinine, being compared in a colorimeter with standard creatinine solutions, after treatment with alkali. Setting the standard at 20 on the colorimeter and using the above method the creatinine may be read directly from the table in milligrams per 100 c.c. of blood.

The sugar determination is made according to the method mentioned and the standard used is 3 c.c. of a solution of pure glucose solution containing 200 mg. of glucose per liter of saturated picric acid. The same amount of standard in this case must be treated with alkali and heated exactly as the unknown. With the standard set at 20 the results in percentage of glucose may be read in Table IV.

TABLE III—CREATININE

STANDARD SET AT 20	0.2 MG. PER 100 C.C. STANDARD	0.4 MG. PER 100 C.C. STANDARD	0.6 MG. PER 100 C.C. STANDARD	2.0 MG. PER 100 C.C. STANDARD	4.0 MG. PER 100 C.C. STANDARD
Unknown Reading					
10	2.00	4.00	6.00	20	40
11	1.82	3.64	5.45	18.2	36.4
12	1.67	3.34	5.0	16.7	33.3
13	1.54	3.08	4.6	15.4	30.8
14.0	1.43	2.86	4.29	14.3	28.5
14.5	1.38	2.76	4.14	13.8	27.6
15.0	1.33	2.66	4.00	13.3	26.6
15.5	1.29	2.58	3.87	12.9	25.8
16.0	1.25	2.50	3.75	12.5	25.0
16.5	1.21	2.42	3.64	12.1	24.2
17.0	1.17	2.34	3.53	11.7	23.5
17.5	1.14	2.28	3.43	11.4	22.8
18.0	1.11	2.22	3.33	11.1	22.2
18.5	1.08	2.16	3.24	10.8	21.6
19	1.05	2.10	3.16	10.5	21.0
19.5	1.02	2.04	3.08	10.2	20.4
20	1.00	2.00	3.0	10	20
20.5	0.97	1.94	2.92	9.7	19.4
21	0.95	1.90	2.85	9.5	19.0
22	0.91	1.82	2.73	9.1	18.2
23	0.87	1.74	2.60	8.7	17.4
24	0.83	1.66	2.50	8.3	16.6
25	0.80	1.60	2.40	8.0	16.0
26	0.77	1.54	2.30	7.7	15.4
27	0.74	1.48	2.22	7.4	14.8
28	0.71	1.42	2.14	7.1	14.2
29	0.69	1.38	2.07	6.9	13.8
30	0.67	1.34	2.0	6.7	13.4

Urea is determined according to the method of Marshall<sup>3</sup> modified by Van Slyke.<sup>4</sup> Two c.c. of blood are diluted with water to about 10 c.c. and 25 mg. of pure urease added (about a gram of freshly ground soy bean may be used instead) and the ammonia formed aerated into 10 c.c. of 0.01 N acid. The last traces of ammonia are brought over by making the blood alkaline with 10 per cent sodium carbonate. Methyl red is placed in the acid into which the ammonia is aerated, so that it is possible to see if the 10 c.c. of 0.01 N acid can take care of all the ammonia formed. The methylene blue is added and the excess of acid titrated with 0.01 N alkali. The results in mg. per 100 c.c. are read from the table.

As was the case in the nonprotein nitrogen the urea will be very high in uremic cases, and the ammonia formed will have to be aerated into 10 c.c. of 0.05 N acid and titrated with 0.05 N alkali, and the results read on Table VI.

Uric acid is determined by a modification of Benedict's<sup>5</sup> method. 10 c.c. of blood are coagulated by adding them to 50 c.c. of boiling 0.01 N acetic acid filtered and boiled down to a small volume and 15 c.c. of alumina cream added and filtered. The filtrate is concentrated to a few cubic centimeters and washed into a centrifuge tube and 20 drops of ammoniacal silver lactate added



TABLE IV—SUGAR IN BLOOD

STRENGTH OF STANDARD 200 MG. PER 1000 C.C.	
Colorimeter Reading	Percentage of Sugar in Blood
7.	0.286
7.5	0.267
8.	0.25
8.5	0.235
9.	0.222
10.	0.202
11.	0.182
12.	0.167
13.	0.154
14.	0.143
15.	0.133
16.	0.125
17.	0.117
18.	0.111
19.	0.105
20.	0.10
21.	.095
22.	.091
23.	.087
24.	.083
25.	.080
26.	.077
27.	.074
28.	.071
29.	.069
30.	.067
31.	.064

TABLE V—UREA

10 C.C. ACID N/100			2 C.C. BLOOD.				MG. UREA PER 100 C.C. OF BLOOD			
c.c.	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0	150.0	148.5	147.0	145.5	144.0	142.5	141.0	139.5	138.0	136.5
1	135.0	133.5	132.0	130.5	129.0	127.5	126.0	124.5	123.0	121.5
2	120.0	118.5	117.0	115.5	114.0	112.5	111.0	109.5	108.0	106.5
3	105.0	103.5	102.0	100.5	99.0	97.5	96.0	94.5	93.0	91.5
4	90.0	88.5	87.0	85.5	84.0	82.5	81.0	79.5	78.0	76.5
5	75.0	73.5	72.0	70.5	69.0	67.5	66.0	64.5	63.0	61.5
6	60.0	58.5	57.0	55.5	54.0	52.5	51.0	49.5	48.0	46.5
7	45.0	43.5	42.0	40.5	39.0	37.5	36.0	34.5	33.0	31.5
8	30.0	28.5	27.0	25.5	24.0	22.5	21.0	19.5	18.0	16.5
9	15.0	13.5	12.0	10.5	9.0	7.5	6.0	4.5	3.0	1.5

TABLE VI—UREA

10 C.C. N/20 ACID.			2 C.C. BLOOD.				MG. UREA PER 100 C.C. OF BLOOD			
c.c.	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0	750.	742.5	735.0	727.5	720.	712.5	705.	697.5	690.	682.5
1	675.	667.5	660.0	652.5	645.	637.5	630.	622.5	617.	609.5
2	600.	592.5	585.0	577.5	570.	562.5	555.	547.5	540.	532.5
3	525.	517.5	510.0	502.5	495.	487.5	480.	472.5	465.	457.5
4	450.	442.5	435.0	427.5	420.	412.5	405.	397.5	390.	382.5
5	375.	367.5	360.0	352.5	345.	337.5	330.	322.5	315.	307.5
6	300.	292.5	285.0	277.5	270.	262.5	255.	247.5	240.	242.5
7	225.	217.5	210.0	202.5	195.	187.5	180.	172.5	165.	155.5
8	150.	142.5	135.0	127.5	120.	112.5	105.	97.5	90.	82.5
9	75.	67.5	60.0	52.5	45.	37.5	30.	22.5	15.	7.5

The precipitate formed is silver urate and is centrifuged off and dissolved in KCN and made alkaline with  $\text{Na}_2\text{CO}_3$  and uric acid reagent added and diluted to a definite volume and compared in a colorimeter with a uric acid standard, a solution 5 c.c. of which gives 1 mg. of uric acid. For routine work the standard is set at 20. The colorimetric reading on the table gives milligrams of uric acid per 100 c.c.

TABLE VII—URIC ACID

DILUTION OF UNKNOWN VOLUME OF BLOOD COLORIMETER READING	25 50 10 20	50 100 10 20	25 20
10.0	10	20	5.0
10.5	9.5	19.0	4.7
11.0	9.1	18.2	4.5
11.5	8.7	17.4	4.3
12.0	8.3	16.7	4.1
12.5	8.0	16.0	4.0
13.0	7.7	15.4	3.8
13.5	7.4	14.8	3.7
14.0	7.1	14.3	3.5
14.5	6.9	13.8	3.4
15.0	6.7	13.3	3.3
15.5	6.4	12.9	3.2
16.0	6.2	12.5	3.1
16.5	6.0	12.1	3.0
17.0	5.87	11.75	2.9
17.5	5.7	11.4	2.8
18.0	5.55	11.1	2.75
18.5	5.4	10.8	2.7
19.0	5.2	10.5	2.6
19.5	5.12	10.25	2.55
20.	5.0	10.	2.5
21.	4.7	9.5	2.3
22.	4.5	9.1	2.2
23.	4.3	8.7	2.1
24.	4.1	8.3	2.05
25.	4.0	8.0	2.0
26.	3.8	7.7	1.9
27.	3.7	7.4	1.85
28.	3.6	7.1	1.8
29.	3.4	6.9	1.7
30.	3.3	6.7	1.6

## BIBLIOGRAPHY

- <sup>1</sup>Greenwald, I.: Jour. Biol. Chem., 1915, xx, 629.  
<sup>2</sup>Myers, V. C., and Bailey, C. V.: Jour. Biol. Chem., 1916, xxiv, 147.  
<sup>3</sup>Marshall, E. K., Jr.: Jour. Biol. Chem., 1913, xv, 487.  
<sup>4</sup>Van Slyke, D.D., and Cullen, G. E.: Jour. Biol. Chem., 1914, xix, 211.  
<sup>5</sup>Benedict, S. R.: Jour. Biol. Chem., 1915, xxi, 61.

## TWO SUGGESTIONS OF APPARATUS FOR THE TEACHING LABORATORY\*

1. A DEVICE FOR THE DETERMINATION OF TIME OF MUSCULAR CONTRACTION AND RELAXATION.
2. AN AUTOMATIC KEY.

BY ARDREY W. DOWNS, M.D., AND GEORGE HAYS, M.D., MONTREAL, CANADA

THE first apparatus described in this paper (Fig. 1) is a simple contrivance designed to give a more accurate measurement of the time occupied by the contraction and by the relaxation of a muscle than does the mechanism usually employed. It consists of a rectangular piece of metal 7 cm. long and  $1\frac{1}{2}$  cm. square placed vertically with a metal rod 15 cm. long and 7 mm. in diameter

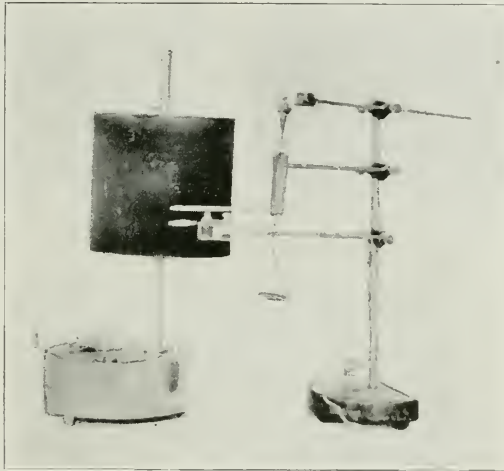


Fig. 1.

attached at right angles near one end to serve as a support. This rod may be clamped to the ordinary stand. Through the center of the vertical block from end to end is drilled a round hole eight millimeters in diameter, and in this a small metal disc, or piston, is accurately fitted. To the upper surface of this disc is fastened a wire for the attachment of the muscle, and to its lower surface is fastened another wire for the suspension of a scale pan. A writing lever is secured to the side of the disc and allowed to project through a slot cut through the cylinder wall from top to bottom. This permits the disc to be moved up and down in its containing cylinder. The muscle may be held in any suitable clamp, in this case a muscle clamp of the type made by the Harvard Apparatus Company.

The muscle lever ordinarily used to record a muscle curve is the radius of a circle and when lifted by the contracting muscle its writing point describes the

\*From the Laboratory of Physiology, Medical Department, McGill University, Montreal, Canada.

arc of a circle. It is obvious that in making a tracing of a muscle curve on a revolving drum the apex of the curve is moved backward, that is, away from the point at which the curve began, because of two factors; the movement of the surface of the drum past the writing point and the arc drawn by the muscle lever when elevated. The second of these factors adds to the apparent length of the period of contraction an appreciable length of time due entirely to

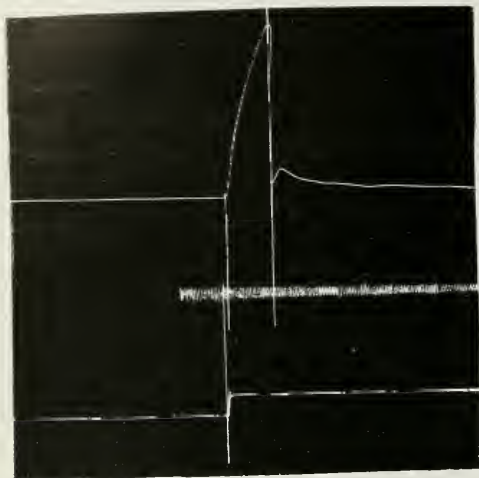


Fig. 2-A.

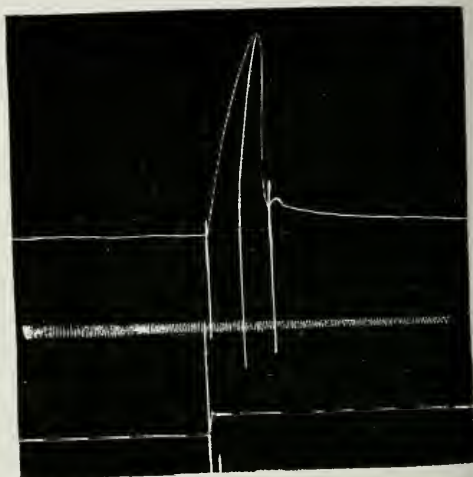


Fig. 2-B.



Fig. 3.

the method of operation of the apparatus. In some cases the arc formed by the writing point is so great as to inscribe a muscle curve in which the apex is behind the point where the lever returns to the base line; i. e., the point indicating the termination of the period of relaxation. In such a case the error is obvious, but in every muscle curve drawn by this method there is an error due to the factor described. It is claimed that this error may be eliminated by allowing the writing lever to inscribe its arc from the apex of the



muscle curve to the abscissa line with the drum stationary, and computing the time from the beginning of the curve to the intersection of this arc with the abscissa line as the true period of contraction. The objections to this method are that it is inconvenient, is apt to be inaccurate, and is, therefore, unscientific.

Two muscle curves exemplifying the foregoing statements are shown in Fig. 2. In Fig. 2*A* is seen a muscle curve ruled with vertical lines from the beginning of the curve, the apex, and the point where the lever returns to the base line, to the time record. It is unnecessary to point out the inaccuracy. In Fig. 2*B* a similar curve is corrected by drawing the arc from apex to base line and then ruling the usual vertical lines. This curve still fails to show the proper relationship between time of contraction and time of relaxation.

In Fig. 3 is shown a muscle curve inscribed by our apparatus and ruled



Fig. 4.—One-half actual size.

in the usual manner. In using this device inaccuracies such as may occur by the method just described are impossible. The writing point can move only in a vertical direction and, therefore, the section of the drum that passes the writing point during the contraction and also during the relaxation of the muscle must represent only the time during which the lever was being raised by the contraction of the muscle or allowed to fall by its relaxation. There is, of course, no magnification as with the usual muscle lever, but the ordinary muscle (in this case the gastrocnemius of a frog) with proper adjustment of load and strength of stimulus will give a contraction curve sufficiently high.

The second suggestion that we wish to make is a simple key which can be

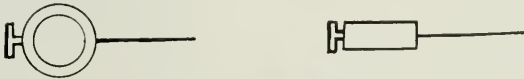


Fig. 5.—One-half actual size.

easily made and attached to the Harvard kymograph. It is so constructed that the primary electric current is broken at any one desired point on the circumference of the drum and is always broken at exactly the same point with each succeeding revolution. Our object in making this key was to secure an arrangement that could be made easily and cheaply, so that it would be suitable for use in a teaching laboratory, which would enable the student to obtain a break shock at identically the same point every time the drum revolved. It is unnecessary to point out the advantages of such a device in recording a series of contractions of a voluntary muscle to show the changes in the contraction curve as the muscle becomes fatigued, or in recording the effect of load upon the contraction of such a muscle. It is not suggested that this key is any better than, or even so good as, automatic keys in use in various laboratories; but

it is intended to offer something which may be helpful to those who are using the Harvard kymograph and have felt, as have the authors, the need of some such attachment.

As may be seen from Fig. 4 the apparatus consists of a hard rubber base  $4\frac{1}{2}$  cm. long and 2 cm. wide which is attached to the upper surface of the base of the kymograph by two screws passing through the vulcanite block at two corners and entering holes drilled through the metal plate forming the upper surface of the kymograph base. In this hard rubber block two binding posts are fastened for the attachment of wires of the primary circuit. One binding post is shorter than the other and carries a metal bar which can be allowed to rest on the taller binding post and which can be swung upward and backward away from the post on which it rests. To accomplish this it is hinged loosely

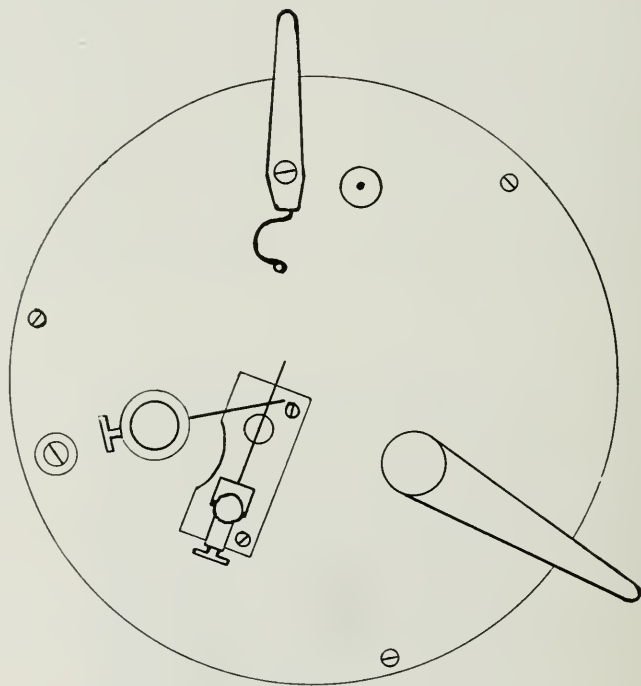


Fig. 6.—One-half actual size.

by a horizontal rod passing through a hole drilled through the shorter of the two posts at right angles to the line joining the binding posts. The only portion of the device remaining to be described consists of a ring (Fig. 5) which encircles the brass sleeve that supports the drum. This ring is held in position by a set screw and carries a rod three centimeters long projecting horizontally. This rod acts as a striker, raises the bridge connecting the two binding posts and carries it onward until it passes beyond the vertical position and drops down and away from the first post. As soon as the bridge is raised the primary circuit is broken and before the drum has completed its revolution ample time is afforded the experimenter to close the short-circuiting key, make the current in the primary circuit by swinging the bridge over into position so as to connect

the two binding posts, and open the short-circuiting key. When this is done the next revolution of the drum gives a break shock at exactly the same point as before. Or, if more convenient, the operator may wait until the curve has been recorded, stop the drum, and reset the key. One great advantage possessed by such a key as we have described is that after the contact has been broken it makes no difference how often the drum revolves there can not possibly be any further stimulation of the preparation until the experimenter closes the key. In our experience this last feature is particularly valuable in the case of students.

Fig. 6 shows the position of the key upon the upper surface of the base of the kymograph.

## A SIMPLE MOUNTING FOR THE CARBON DIOXIDE APPARATUS OF VAN SLYKE\*

By WITHROW MORSE AND L. L. LANDENBERGER, CHICAGO, ILL.†

THE accompanying figure illustrates a simple method of mounting the apparatus of Van Slyke for carbon dioxide determinations as used in this laboratory.† The larger apparatus is shown here, but the same method is applicable

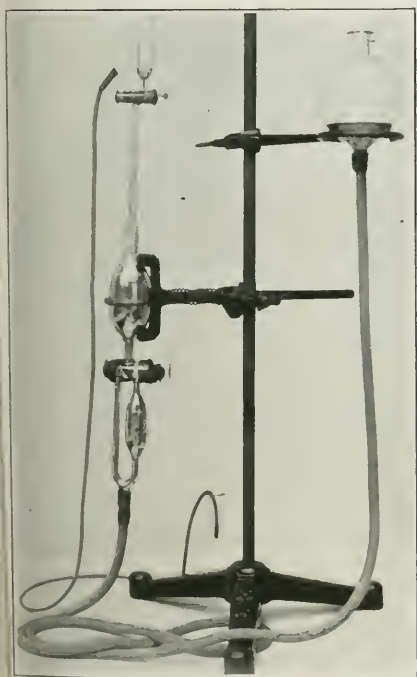


Fig. 1.

to the microapparatus. The 50 c.c. bulb of the apparatus sits in a clamp‡ (Fig. 2) which has four jaws generously supplied with thick-walled rubber tubing and the bulb is held in place by means of a hook operated by a spiral spring at its base, which draws the hook into firm contact with the bulb. The hook is covered, likewise, with rubber tubing.

The leveling bulb is supported by an ordinary ring fastened to the same ringstand, which is used to hold the apparatus proper. From the sides of the ring, two inches of metal are cut away for the purpose of admitting the leveling bulb.

In the manipulation of the apparatus during a determination, it is necessary to



Fig. 2.

\*Van Slyke, D.D.: Jour. Biol. Chem., June, 1917.

†From the Nelson Morris Memorial Institute for Medical Research, Michael Reese Hospital, Chicago.

‡This clamp is patented and made by Wm. Gaertner & Co., 5345 Lake Park Ave., Chicago, and is readily adapted to many other uses in the laboratory.

remove the apparatus from its support. This is simply done by slipping the hook from the bulb. There are no screws necessary.

It is important that the two-way stopcock at the bottom of the 50 c.c. bulb be held firmly in place. This may be done by means of heavy rubber bands, but this obscures the apertures in the stopcock. By means of a piece of wood fitted in a fork around the neck of the stopcock and held firmly against the tube by means of zinc oxide adhesive or a rubber band, displacement of the stopcock and consequent loss of mercury is rendered impossible.

## NOTE ON A UNIFORMLY SATISFACTORY METHOD OF COLLECTING URINE SEPARATELY FROM EACH URETER IN ACUTE EXPERIMENTAL WORK (DOGS)\*

BY A. B. LUCKHARDT, PH.D., M.D., CHICAGO, ILL.

IT seemed desirable to record briefly a method of collecting urine separately from each ureter which is better than the procedures in common use in acute experiments inasmuch as it is quite satisfactory even in the hands of an amateur.

It will be generally conceded, I believe, that all methods of collecting urine involving cannulas tied in the ureters have this annoying difficulty that the ureter from the start or soon after suffers compression from the abdominal

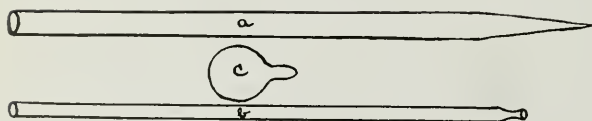


Fig. 1.—(a) Steel-tipped brass trocar large enough to admit ureteral cannula (b). The wood fiber sphere (c) will be found useful in pushing trocar through soft tissues.

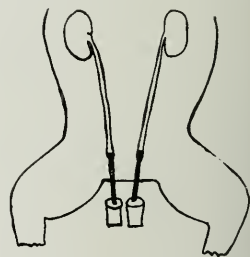


Fig. 2.—Shows the artificial posterior extension of dog's ureters by cannulas introduced into the ureters and held in place by a method described in the text.

wall or surrounding structures or obstruction by a kinking of the ureter at the point of insertion of the cannula, especially when an attempt is made to lead out the collecting cannulas through an incision in the anterior abdominal wall. Particularly is this true if the cannulas are introduced into the ureter near the bladder, the point of election, since insertion near the pelvis of the kidney invites a reflex anuria.

The method I have devised is based on three mechanical physiologic considerations of practical importance: 1. To isolate the ureters through a small abdominal incision just above the symphysis of the pubis. 2. To introduce

\*From the Hull Physiological Laboratory of the University of Chicago.



cannula in such a manner that it will be in line throughout its length with the ureter into which it has been inserted. 3. To maintain the cannula securely in this position throughout the period of experimentation; for everyone knows that very slight pressure on the ureter or kinking of it suffices to stop an otherwise brisk flow of urine.

To accomplish the latter two ends I make use of trocar of brass tubing provided with a very sharp steel tip (Fig. 1A) sufficiently large to admit a glass cannula whose tip is blunt (Fig. 1B). The trocar is about 15 cm. long with an internal bore of 6-7 mm. The glass cannulas are somewhat longer, the length varying with the size of dog experimented upon. The urinary bladder having been emptied of the contained urine and fecal masses removed through the anus by manual compression, through a laparotomy incision just above the symphysis two small skin incisions are made, each one medial to the tuberosity of the ischium. A wood-fiber sphere having a small projection which fits snugly in the open end of the brass trocar (Fig. 1C) is attached to the latter. The trocar is grasped firmly in one hand with the wood-fiber plug in the center of the palm; the tip of the trocar is inserted into one of the skin incisions near the tuberosity of the ischium and then pushed through the perineum and pelvis under the symphysis on the pubis so that the trocar, when its tip appears above the brim of the true pelvis lies in a direct line with the ureter. The wood-fiber sphere is disengaged from the trocar. A ureteral cannula (Fig. 1B) is pushed up into the trocar (tip first) as far as it will go. The trocar is removed by pulling it out of the abdominal incision leaving in its place the ureteral cannula in exact line with the ureter. The cannula is tied into the ureter in the usual way. In short, the ureter is artificially extended backwards and to the exterior by a cannula which lies *in line* with the ureter and is held there firmly by the tissues surrounding it. (Fig. 2.)

In plunging the trocar through the tissues it is, of course, essential that the tip be directed properly so that it will subsequently lie in the proper position. The index finger of the other hand will be found useful as a guide.

If the introduction of the long cannula into the ureter is found too difficult, a small cannula may be inserted into the ureter, a longer piece of glass tubing brought in apposition with it by means of the trocar, and the two then joined by a piece of thick-walled rubber tubing.

After the cannulas are in place they are freed from air by displacing the latter with physiologic salt solution or water contained in a bent Pasteur pipette inserted high up into the neck of the ureteral cannulas.

For convenience in introducing the trocars and in collecting urine it is desirable to raise the dog's pelvis from the animal board by a two-inch wooden block.

Since the ureters converge towards the bladder, it would possibly be still better to push the trocar from the left skin incision to the right brim of the true pelvis and from the right skin incision to the left brim of the pelvis. The ureteral cannulas when inserted would then cross in the median line under the symphysis. But I have not found it necessary to cross the cannulas to conform with this strictly theoretical requirement.

# *The Journal of Laboratory and Clinical Medicine*

VOL. III.

JUNE, 1918

No. 9

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.  
Ann Arbor, Mich.

## ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D.	-	-	ST. LOUIS
HANS ZINSSER, M.D.	-	-	NEW YORK
PAUL G. WOOLLEY, M.D.	-	-	CINCINNATI
FREDERICK P. GAY, M.D.	-	-	BERKELEY, CAL.
J. J. R. MACLEOD, M.B.	-	-	CLEVELAND
ROY G. PEARCE, M.D.	-	-	CLEVELAND
ROGER S. MORRIS, M.D.	-	-	CINCINNATI
GERALD B. WEBB, M.D.	-	-	COLORADO SPRINGS
E. E. SOUTHARD, M.D.	-	-	BOSTON

Contents of this Journal Copyright, 1918, by The C. V. Mosby Company—All Rights Reserved  
Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

## EDITORIALS

### *An Explosive Epidemic of Influenzal Disease at Fort Oglethorpe*

MAJOR SOPER makes a report upon the above-named epidemic of which the following is an abstract:

A disease strongly resembling influenza became prevalent in the Oglethorpe camps about March 18, 1918. It soon assumed pandemic proportions. Within two weeks every organization in Camp Forrest and the Reserve Officers' Training Camp was affected. It seems to have visited only a part of Camp Greenleaf. The War Prison barracks were not invaded. After about three weeks the epidemic subsided rapidly. The number of cases sent to hospital or to quarters was 1,468 in a total strength of 28,586. Owing to the fact that many cases were not severe, the total number of officers and men attended can not be given; an estimate based on replies to a circular letter of inquiry to the several organizations of the study of the records of the hospitals indicates that not less than 2,900 cases have occurred in Chickamauga Park.

The attention of the Camp Surgeon's Office was called to the existence of this disease on March 18, at which time the writer saw a number of men appear at sick call in the 51st Infantry, suffering with the disease which the regimental surgeons were unable to diagnose. The symptoms were as follows: Headache pain in the bones and muscles, especially the muscles of the back, marked pros

tration, fever (sometimes as high as  $104^{\circ}$ ). Sometimes there was conjunctivitis, coryza, a rash and possibly nausea, recovery taking place in a few days.

In most cases a definite diagnosis was not made at the regimental sick call, but at the receiving ward, when a name was given, it was usually called influenza.

On April 3 it was recommended that steps be taken to ascertain from the several organizations under the supervision of the camp surgeon from other camps in the Oglethorpe region, the essential facts concerning the nature and epidemiological progress of the disease. A circular letter of inquiry upon these lines was sent out on April 4 to all organizations. Replies to this questionnaire indicated that the disease was first noticed in epidemic form on March 18 in the 51st Infantry. In some organizations all cases were sent to hospital; in others their cases were treated in quarters or in the regimental infirmary. This does not mean that the disease was more severe in some organizations than in others. Difference in the disposition of the sick depended not so much upon the severity of the case as upon the local facilities for dealing with them. Records show that there were twice as many patients dealt with in quarters as in hospitals and there were probably twice as many cases existing as were carried on sick report.

The replies were unanimous in stating that the disease was not restricted to recruits, nor to men who had already experienced or failed to experience an attack of measles, German measles or scarlet fever. One attack did not protect against another.

In all the organizations the epidemic was first located in companies before it became general. In many instances a large proportion of the men were affected. According to the registrar of the Base Hospital, fully one-half of the total number of patients in the hospital had the disease. The rate at which the epidemic progressed made it impossible to trace its path, if indeed it followed any.

The incubation period was short, usually not over one or two days. Instances were found in which men isolated in quarantine were attacked apparently through contact with those who brought them food or approached them for other reasons. At the Post Hospital a number of surgical cases in tents were infected, apparently by an orderly. In Co. B, of the 15th Machine Gun Battalion, which was in quarantine on account of scarlet fever, nearly every man present was attacked. In Company A, 52nd Infantry, a company which was quarantined, the men appeared to be protected by reason of their isolation.

Some organizations suffered more than others for no apparent reason. The 52nd Infantry had the most and the 54th Infantry the fewest cases. In the 52nd Infantry and the 15th Machine Gun Battalion, one in every seven of the officers and men was on sick report. In one ambulance company the proportion was one to six, while in others it ran as low as one to twenty-two.

Upon the recommendation of Major Soper, a board was created on April 10 to determine the nature of the disease causing the unusually large number of admissions to the Post Hospital with the diagnosis, "fever, type undetermined," "influenza," etc. This board has not yet finished its work. The appearance of the epidemic in various organizations of the 6th Division was so nearly simultaneous as to suggest that a common cause was operating or that the disease was

spreading with great rapidity. The first recorded notice of the disease was in the 51st Infantry on March 18. Three days later it was recognized in the 52nd Infantry and the 15th Machine Gun Battalion. On March 25, the 16th, 17th, 18th, and 19th Machine Gun Battalions encountered it. The 11th Infantry was infected on March 26, and the Camp Quartermaster's Office on March 28; the 11th Cavalry on March 30, and the 53rd and 54th Infantry on April 1. Camp Warden McLean seems not to have been visited by the epidemic until April 7; by the 9th, 200 cases of more or less severity had occurred there.

The possibility that the disease which has just become epidemic has long existed in these camps in sporadic form, but has not hitherto attracted notice because of its infrequency, has received careful consideration. At first there seemed much to recommend this theory. Clinicians on the hospital staff claimed to have seen the disease at times since the summer of 1917. They say that it became rather prevalent in September. It is believed by some that the germ of influenza, like that of some other diseases, is constantly present in every large community; that it is not always virulent, but under certain conditions it acquires increased virulency.

If the organism which has caused the present epidemic has long been in existence in the Oglethorpe camps, it is not apparent why it has suddenly become so active. It would seem that the conditions for its epidemic prevalence have existed for a long time. Recent weather conditions, uncomfortable as they have been, have not been so disagreeable as they were in January. The theory that the explosion is of local origin needs a better explanation than can apparently be given it.

Studies by Major Soper have been made to ascertain whether the state of the weather was in any way accountable for this epidemic. This inquiry covers the period from March 1 to April 13, approximately three weeks before the epidemic began and more than three weeks later; 44 days in all. For the six weeks' period there was less than half the amount of sunshine which was possible. On 11 days there was 1/5 or less of sunshine which would have occurred had the days been perfectly clear. On 9 days the sun was obscured during 9 per cent of the time. The mornings, evenings and nights were misty; the day was generally well advanced before the sun appeared. The days were fairly mild, but nights and mornings were damp and chilly. The highest temperature on any day was 82. It was warmer than 75 degrees seven times; over 65 degrees twenty-five times, and over 55 degrees thirty-eight times, or nearly every day. The mean daily temperature was always under 75 degrees or less, about as often as it was higher than that figure. The minimum daily temperature was 45 degrees, or less, half the time. On 11 days the mercury fell to 40 or under. These temperatures would not have been in the least uncomfortable had the weather been less humid. There was a sudden drop in temperature on the 9th of March. On the evening of that day the thermometer fell from 68 degrees at 7:00 P.M. to 38.8 at 7:00 A.M., or 30 degrees over night. The fall was followed by a rise to 76 degrees and then by a drop of 14 degrees to 62. On March 14, the temperature fell from 71 to 60 in ten minutes. Another fall of 9 degrees at this time of from 72 to 63, occurred on the morning of April 3.

The relative humidity in the morning at 7:00 o'clock was nearly 60 per cent or over. For nearly three-fourths of the time it was 70 or more. On 8



out of the 44 days the humidity reached 80 or more in the morning. The humidity was somewhat less in the evening. It was 70 per cent or more about one-fourth of the time. About one-third of the time it was 60 or more. At noon a humidity of 60 per cent occurred nearly one-half the time, 70 per cent over one-third of the time and 80 per cent about one-fifth of the time. These humidities were not remarkable in themselves. Considered in connection with the temperature they were of a sort to aggravate respiratory affections.

The weather was cloudy, damp and chilly. It was not cold nor wet, but the nights and especially the mornings were decidedly damp and uncomfortable. The difference between the air indoors and out was marked. Drafts were particularly noticeable.

Reviewing these facts about the weather, it can not be said with certainty that the conditions of temperature and humidity have had much to do with the epidemic, nor can it be denied that they played an important part in predisposing the troops to attack. Obviously the weather conditions have led the men to gather indoors where they have been especially exposed to infection from one another, and it has had a chilling effect which is an important factor in the progress of all respiratory infections.

An inquiry was made to ascertain whether the troops were exposed to an unusual extent to the weather, to excessive fatigue, or in any other way before or after the epidemic started. This line of investigation brought no suggestive information to light.

No exceptional prevalence of influenza or other similar infectious disease has existed in Chattanooga or in the extra cantonment zone. An effort was made to shed light on the identity of the disease by studying the record of other cases of sickness which had been sent to the hospital under the designations of "fever, type undetermined" and "influenza" during the six months preceding the epidemic. These designations have never been definite; they have seldom been based on conclusive evidence at the hospital. Bacteriological examinations have seldom proved the influenza bacillus to be the causative agent of the cases called "influenza." From time to time this bacillus had been found in the secretions of the nose and throat of troops, but has not been proved to be the cause of any large amount of sickness. "Fever, type undetermined" and "influenza" have been merely convenient expressions by which to designate cases of an indeterminate sort which demanded treatment and had to be called something.

Of a series of 161 cases of "fever, type undetermined" sent to the hospital before the present epidemic broke out, 59 turned out to be bronchitis and 41 pneumonia; 61 were called "influenza." In some instances "fever, type undetermined" has been found to be measles, scarlet fever, otitis media and meningitis. Out of 189 cases diagnosed "influenza" at the regiments, 150 turned out the hospital to be some other disease.

It is probable that the epidemic disease was recently brought to these camps. If it is genuine influenza, and the epidemiological features no less than the leading symptoms seem to point to that disease, there is here offered the most reasonable explanation of the outbreak which is now possible. No other disease spreads so fast or is so prostrating, considering its symptoms. Influenza may be nearly explosive in character, it spreads as rapidly as personal

communication permits. Personal contact is intimate in the Oglethorpe camps, especially between men in companies and regiments. To some extent the regiments keep separate, but there is a general mixing at places of amusement in camp and Chattanooga. It is worthy of remark that the regular Officers' Training Camp is an organization which mingles but little with others in the Oglethorpe camps, and that here the epidemic was late in appearance. The same may be said of a part of Camp Greenleaf. The War Prison Camp is entirely separate and escaped infection. The epidemic seems to have burned out for want of suitable material, probably with the gradual but rapid decrease in virulence.

Reviewing the whole subject, it may be said that an epidemic of influenza disease became prevalent in the Oglethorpe camps toward the latter part of March, 1918. The identity of the disease has not been positively determined after nearly a month of observation. It may have been an outburst of a form of sickness which has long existed in sporadic form in these camps. It may have been brought to the camps from outside. The weight of evidence is in favor of the latter theory. Such laboratory evidences as may be available up to this point will be reserved for the initial report of the board which was created on April 3 in order to determine the identity of the disease.

It is a highly infectious disease with a short period of incubation. The weather has encouraged the epidemic, but is not apparently responsible. The disease is respiratory in type, with a strong resemblance to influenza in some of its most characteristic symptoms, as note the fever, pain in the back and legs and great prostration.

The cause of the rapid spread undoubtedly lies in the great infectivity of the causative agent, its short period of incubation and the intermingling of the troops. One thing seems clear, the disease could never spread unless the buccal or nasal discharge of the sick got into the mouths or noses of susceptible persons. The nature of the epidemic seems to show the extent to which the interchange takes place under the conditions which surround these troops.

—V. C. V.

### *Epidemic Meningitis and Its Treatment*

THE belief that epidemic cerebrospinal meningitis is essentially a primary infection of the meninges has been generally accepted despite the very obvious clinical symptoms which would seem to point in a different direction, and despite the fact that a consideration of the route by which the organisms must follow in order to reach the meninges would seem to suggest the probability that the blood stream would be primarily involved. When symptomatology and pathogenesis are taken together the logic is entirely in favor of the primary septic nature of the disease.

It is just as Herrick<sup>1</sup> says,—“the meningeal aspect of meningococcus infection has usurped such a prominent place in the commonly accepted ideas of the nature of epidemic cerebrospinal meningitis, that important facts have been kept in the background. The very name of the disease and of the causative

organism have given undue prominence to a single manifestation of the condition." The conception that meningococcus infections are primarily septic conditions and that the meningitis is a secondary affair has been expressed, but, as Herrick says, it has never been adopted as a working basis for early diagnosis and treatment. This is true to such a degree that the premeningitic stage of the disease,—the period of the disease prior to the time when localization occurs,—has been practically disregarded. It is upon this stage of the disease and upon treatment during it that Herrick dwells in his account of his experience with 208 cases at Camp Jackson.

The facilities for the study of these cases at Camp Jackson, while rudimentary in the extreme in some respects, were in other ways ideal. It was realized by the Division Surgeon that good results would depend upon early diagnosis and treatment, and therefore he instructed the regimental surgeons to refer at once to the Base Hospital all men complaining of headache, fever, vomiting, or other suggestive symptoms. Such a system would insure not only getting the cases of meningitis at an early stage, but would also make it possible to catch cases of other infections. The result of this order was that a very unusual number of early cases of meningitis were made available for study.

The major results of the study are summarized as follows: The earliest stage of the disease is brief and lasts on an average about 48 hours, during which the symptoms are those of a generalized infection. In some cases this is the only stage and the disease is of the abortive type, or, in exceptional instances of the fulminating type without meningeal symptoms. One-half of Herrick's cases were recognized in this "premeningitic period." In a very large proportion of the cases there was an associated upper respiratory tract disturbance, a phenomenon of such frequent occurrence that, as Herrick, Medlar,<sup>2</sup> Mink,<sup>3</sup> and Thomsen and Wolff<sup>4</sup> have suggested, it is no mere coincidence. Of the early symptoms the skin manifestations are most important. The predominant skin sign is a petechial rash which appears about the shoulder or pelvic girdle if at all, and next in frequency, over the trunk, extremities, face, oral, mucosa and conjunctiva. It appears with astonishing rapidity in crops and in some of the severe cases the patient becomes well dotted within an hour. In the presence such a rapidly developing hemorrhagic rash is diagnostic. Purpura is a feature of fulminating cases.

Next to the rash the most important symptoms are modifications of the reflexes, especially *unequal* enhancement of the deep reflexes. Also slight chills are present and headache is present in 85 per cent of the cases. In the presence of a combination of any two of these suspicious symptoms a lumbar puncture is done and the fluid, even if it is clear, comes out under slight increase of pressure and may show a normal cell count. Long centrifugation followed by careful examination of the sediment will usually reveal a few pairs of the cocci. If examination of the fluid first obtained is not productive, a second puncture is made shortly after it, and in this the organisms will be found. At this very early stage of the disease Baeslack<sup>5</sup> and his associates have found meningococci in the blood stream in 36 per cent of the cases examined.

Herrick describes four types of meningococcic sepsis, as follows:

The abortive type. A mild, systemic disturbance without a local focus of

suppuration. In this the diagnosis depends upon a demonstration of the organisms in the spinal fluid, blood, nasopharynx, or conjunctiva. The importance of cases of this type is chiefly epidemiologic because they are a menace to susceptible contacts.

The ordinary type. In this the symptoms of meningitis develop gradually, unconsciousness is rare, and the course may be prolonged. The chief emphasis is upon the meninges. Hydrocephalus is a danger and the response to intravenous serum therapy is often less prompt than in the more severe cases.

The severe type. In this there are all the evidences of a severe toxemia. The petechial rash appears with great rapidity. Death may occur before metastasis takes place. The response to serum therapy by the intravenous method is, as a rule, prompt. Intraspinous serum treatment alone is usually insufficient. Diagnosis in the premeningitic stage is usually possible, and blood cultures are usually positive.

The fulminating type. In this as a rule meningitis is not present. Purpura is characteristic. Death is usually exceedingly prompt, and serum treatment is commonly ineffective.

Herrick has worked out the following method of procedure as the result of his study of 208 cases: On admission a patient presenting any combination of the very early symptoms is immediately subjected to lumbar puncture. If the fluid is cloudy enough it is removed to reduce the intraspinal pressure to an approximate normal and a less amount of serum is at once allowed to run into the spinal canal. If the fluid is clear no intraspinal injection is made. The fluid is rushed to the laboratory in a thermos container and immediately examined. In the meantime the patient receives a desensitizing dose of serum. One hour later 50-120 c.c. of serum are given by vein, the first 15 c.c. at the rate of 1 c.c. per minute. In cases of ordinary severity this dose is repeated every twelve hours until the temperature becomes normal or until six to eight doses have been given. In severe cases serum is repeated every eight hours until the desired results are produced. For further details the original article should be consulted.

The results of this procedure in treatment are that the total mortality in the 208 cases was 26 per cent. In 129 cases treated by intraspinal methods alone or with but small doses by the veins, the mortality was 31.7 per cent. In 7 cases treated with the larger doses of serum by the intravenous method and with average doses intraspinally the mortality was 16.4 per cent. The mild cases do well with either method. In the severe cases the intravenous method was especially brilliant. Intravenous therapy had the additional value over the older methods in that it reduced complications. Thirty-one per cent of the cases treated by the older method developed complications. In the group receiving the larger intravenous doses the percentage of complications was fourteen

—P. G. W.

#### BIBLIOGRAPHY

- <sup>1</sup>Herrick: Jour. Am. Med. Assn., 1918, lxx, 227; Arch. Int. Med., 1918, xxi, 541.
- <sup>2</sup>Medlar: Jour. Am. Med. Assn., 1918, lxx, 458.
- <sup>3</sup>Mink: Ibid., 1916, lxvi, 463. (Abstract.)
- <sup>4</sup>Thomsen and Wolff: Ibid., 1918, lxx, 498. (Abstract.)
- <sup>5</sup>Baelsack, et al.: Jour. Am. Med. Assn., 1918, lxx, 684.



*Epidemic Bronchitis at Fort Oglethorpe, Georgia*

MAJOR GEORGE A. SOPER, S. C., N. A., has made a report upon epidemic bronchitis at Fort Oglethorpe. The following is an abstract of the same:

Bronchitis has been well-nigh universal in the Oglethorpe group of camps. Within ten days after their arrival newcomers have generally been attacked, the symptoms often being pronounced from the start. Sneezing and coughing are early signs. In the barracks, mess halls, lecture rooms and places of amusement, during November, December and January there was seldom a moment when coughing was not noticeable and continuous. It is estimated that 80 per cent of all the troops were affected. The epidemic abated in February with the advent of warm weather. Laboratory findings do not agree that any single organism has been the cause. Streptococci, staphylococci, influenza bacilli and other organisms found in the noses and throats of healthy people and sometimes associated with disease have been isolated, but not under circumstances which have led to any of these germs being demonstrated as the microbic cause of the bronchitis. The infectious matter has passed from person to person, probably in three ways: First, men have talked with one another at close range, permitting mouth germs to be projected directly into one another's faces. In the second place, there has been a general impregnation of the atmosphere in confined spaces. In the third place, articles handled by the infected have been transmitted to others. In these ways the amount of infectious matter which has passed from person to person must have been large and with lowered resistance, the infection has rapidly spread.

Predisposing and contributing causes existed to some extent. The weather was unfavorable. Changes in the temperature were frequent and marked. The ground was cold, the air damp and the nights cold. Often men did not have dry shoes for weeks at a time. Some spent the entire winter in khaki. The men themselves were ignorant of simple personal precautions which might greatly have lessened their chances of infection. All of the severe cases have gone to the hospital. The universal prevalence is significant both on its own account, and because of the light which it throws upon the spread of other respiratory diseases in these camps. Although most persons regard bronchitis with comparative indifference, it is a disease of much significance. It is the common saying among the troops that the "bronchial cold" which attacks them soon after their arrival remains with them as long as they stay in camp. Its characteristic hard, explosive cough remains after other symptoms have disappeared. For the time it often unfits a man for duty and there are few sufferers whose efficiency has not been impaired by it. The bronchial pneumonias of these camps have been frequent sequelae to bronchitis. Whether bronchitis renders a man especially susceptible to other acute respiratory diseases is a question of much interest. Like pneumonia one attack does not protect against others, but on the contrary seems to predispose its victim to subsequent attacks. It is not at all probable that bronchitis increases resistance to measles, scarlet fever, pneumonia and other such diseases. The part which bronchitis may play in the spread of other respiratory infections gives reason for regarding it as a camp disease of the utmost importance. A carrier of meningitis may be relatively harmless so

long as he is in good health, for then the germs which he harbors are fairly well locked up in his nose and in his throat, but when he experiences an attack of bronchitis he sneezes and coughs and at each paroxysm germs are shot into the air. Therefore, it is reasonable to suppose that measles, scarlet fever and other respiratory diseases have been spread by bronchitic soldiers. It may be asked if the exchange of bacteria from the nasal pharynx is so general, why is it that more sickness has not occurred. The answer probably is that most robust soldiers possess a fair degree of immunity acquired either by one attack as in the case of measles, or by frequent exposure without suffering from the disease.

The bronchitis which has prevailed in these camps may be designated as an acute infectious inflammation affecting in rapid succession downward the nasopharynx, the larynx, trachea, and first and second divisions of the bronchia mucosa. The symptoms are coryza, chilly sensations, hoarseness and soreness of throat, weakness, muscular soreness and slight fever ( $100$  to  $101^{\circ}$ ). In severe cases the temperature may rise to  $103^{\circ}$  with a correspondingly rapid pulse accompanied by substernal soreness and tightness of the chest. At first the cough is dry and unproductive. It frequently occurs in paroxysms causing muscular pain and soreness along the costal margin at the attachment of the diaphragm. On the third or fourth day, as a rule, the cough loosens and expectoration appears. At first it is scanty and mucous, later it is abundant and mucopurulent. Only negative results are obtained on palpation and percussion. On auscultation sibilant rales may be noted, except when resolution begins, when fine and coarse mucous rales are to be heard. The breath sounds are harsh. Bronchitis may be associated with other diseases such as measles, mumps or scarlet fever. When not complicated, recovery occurs at best within a week although a chronic form may supervene and continue for months.

Although chronic bronchitis as seen in the quarantine camps is believed to be a definite entity, it is not always so diagnosed. Cough, elevation of temperature and muscular soreness accompany acute nasopharyngitis and tracheolaryngitis as frequently as bronchitis. A positive diagnosis of bronchitis is impossible unless harshness of breath sounds, sibilant and sonorous rales are audible, or in stage of resolution, fine and coarse mucous rales are heard. Many cases are diagnosed as bronchitis from the symptoms alone.

Pneumonia is not frequently preceded by acute bronchitis, and acute bronchitis rarely terminates in pneumonia except when it accompanies measles. In measles there may be an extension to the terminal bronchi, and the intercommunicating air cells, under which circumstances bronchopneumonia develops.

It is claimed by some that acute bronchitis can frequently be arrested in its early stages by the local application of silver nitrate, 40 grams to the ounce. This solution is applied to the tonsils and erythematous areas in the pharynx. Nasopharyngeal treatment by sprays is also useful by limiting the source of supply of infectious material which is generated in the nasopharynx.

—V. C. V.

## *Procaine and Novocaine Identical*

TO THE EDITOR:

It appears that in certain quarters the attitude is taken that the local anesthetic sold as Procaine is not identical with that marketed as Novocaine. The Subcommittee on Synthetic Drugs of the National Research Council believes it important that this misunderstanding should be corrected and hence offers the following explanation:

The monohydrochloride of para-amino-benzoyldiethylamino-ethanol, which was formerly made in Germany by the Farbwerke, vorm. Meister, Lucius and Bruening, Hoechst A.M., and sold under the trademarked name Novocaine, is now manufactured in the United States. Under the provisions of the Trading with the Enemy Act, the Federal Trade Commission has taken over the patent that gave monopoly for the manufacture and sale of the local anesthetic to the German corporation, and has issued licenses to American concerns for the manufacture of the product. This license makes it a condition that the product first introduced under the proprietary name "Novocaine" shall be called Procaine, and that it shall in every way be the same as the article formerly obtained from Germany. To insure this identity with the German Novocaine, the Federal Trade Commission has submitted the product of each firm licensed to the American Medical Association Chemical Laboratory to establish its chemical identity and purity, and to the Cornell pharmacologist, Dr. R. A. Hatcher, to determine that it was not unduly toxic.

So far, the following firms have been licensed to manufacture and sell Procaine:

The Abbott Laboratories, Ravenswood, Chicago.

Farbwerke-Hoechst Company, New York, N. Y.

Rector Chemical Co., Inc., New York, N. Y.

Calco Chemical Company, Bound Brook, N. J.

Of these, the first three firms are offering their products for sale at this time, and have secured their admission to New and Nonofficial Remedies as brands of Procaine which comply with the New and Nonofficial Remedies standards.

While all firms are required to sell their product under the official name "Procaine," the Farbwerke-Hoechst Company is permitted to use the trade designation "Novocaine" in addition, since it holds the right to this designation by virtue of trademark registration.

In conclusion: Procaine is identical with the substance first introduced as Novocaine. In the interest of rational nomenclature, the first term should be used in prescriptions and scientific contributions. If it is deemed necessary to designate the product of a particular firm, this may be done by writing Procaine-Abbott, Procaine-Rector, or Procaine-Farbwerke (or Procaine [Novocaine brand]).

Julius Stieglitz, Chairman,

Subcommittee on Synthetic Drugs, National Research Council.

EDITOR'S NOTE:

The Federal Trade Commission recommends the use of the official name of the licensed drugs in connection with all written articles and advertisements, and

if the proprietary brand name is to be used, to place this side by side with the official name.

The official names so far adopted by the Federal Trade Commission are:

*Arsphenamine* for the drug marketed as Salvarsan, Diarsenol, and Arsenobenzol, etc.

*Neoarsphenamine* for the drug marketed as Neosalvarsan, Neodiarsenol and Novarsenobenzol, etc.

*Barbital* for the drug marketed as Veronal.

*Barbital-Sodium* for the drug marketed as Medinal and Veronal-Sodium.

*Procaine* for the drug marketed as Novocaine.

*Procaine Nitrate* for the drug marketed as Novocaine Nitrate.

*Phenylcinchoninic Acid* for the drug marketed as Atophan.



571

# The Journal of Laboratory and Clinical Medicine

VOL. III.

ST. LOUIS, JULY, 1918

No. 10

## ORIGINAL ARTICLES

---

### TOLERANCE AND IMMUNITY

---

BY JOHN L. MARCHAND, M.D., PRINSAPOLKA, NICARAGUA, C. A.

VAUGHAN demonstrated some fifteen years since that the *protein poison* was a fact to be reckoned with in immunology; that the effects of this poison on the experimental animal could, under certain conditions, be other than deleteriously toxic. The rather voluminous literature of particularly the last two or three years on, especially, the treatment of typhoid fever, the arthritides and certain skin affections with nonspecific proteins, and with protein precipitating substances, administered in such manner, and in such dosage, as to elicit the maximum toxic reaction compatible with the welfare of the patient, offers undisputable evidence of the correctness of Vaughan's advancements. This authority, however, is either utterly ignored, or, for the most, misquoted when his work is mentioned at all.

Thus Miller,<sup>1</sup> one of the very few who mentions Vaughan's work, which he does in connection with his treatment of the arthritides with typhoid, and other proteins, given intravenously, misquotes him flagrantly when he states that the latter "observed that it was possible to produce in animals a transitory immunity to the colon bacillus by using peptone and egg-albumin, and that this immunity was apparently equal to that following immunization by the colon bacilli."

Now, while it is quite true that Vaughan did make use of peptone, egg-albumin and colon bacilli, these different proteins were employed in this instance merely for the purpose of extracting their *cell poisons* and for obtaining their *cell residues*. *It was with these poisons and residues, and not with the proteins themselves, that the series of experiments, undoubtedly referred to by Miller, were conducted.*<sup>2</sup> It is also true that, a few years previously, attempts had been made to produce an immunity with the cellular substance of the colon

bacillus; but this preparation proved so toxic, and the results of its administration so evanescent, that it was concluded that the capability of the animal to bear increased doses "was not sufficiently marked-to be designated by the term immunity, and it was decided to recognize it as an increased tolerance."<sup>3</sup> No comparative studies with the other proteins named above, however, were made at this time; but it is of great interest to note that it was the then unrecognized phenomena of sensitization which principally interfered with the drawing of definite conclusions from this earlier work.

As a result of this later work, however, very definite conclusions were drawn. It was found that the cell poisons sensitized neither to themselves nor to the proteins from which they were derived, but that they did produce a certain tolerance, when introduced parenterally into the experimental animal, in properly gaged and properly spaced doses, which enabled these animals to withstand from two to three times the dose invariably fatal to the untreated animal; and, furthermore, it was clearly demonstrated in this series of experiments that animals thus treated with either the cell poison of the colon bacillus, with that from peptone or with that from egg-albumin acquired, in addition to this tolerance, a certain degree of immunity to inoculation with the living germ which enabled them to survive from two to four times the ordinarily fatal dose of the virus.

Quite in contrast with the cell poisons the cell residues proved to be ideal sensitizers, but to produce no tolerance. Animals sensitized with the cell residue of the colon bacillus acquired an immunity to inoculation with that virus which enabled them to withstand *at least eight times* the dose invariably fatal to the untreated animal; *but animals sensitized with the residues of peptone or egg-albumin showed no immunity to such inoculations.*

Tolerance, therefore, is designated as a nonspecific phenomenon, in that the tolerance produced by one protein poison is a tolerance to other protein poisons as well; and the transitory immunity accompanying, or conferred by, tolerance is equally nonspecific.

Sensitization, on the contrary, is regarded as a specific phenomenon, in that the sensitization produced by the sensitizing portion of one protein is a sensitization to that protein alone; and the immunity due to sensitization, *in every way superior to that due to tolerance*, is also specific.

In this series of experiments two very significant facts are to be noted:

1. In the poison immune animal, that is, in the animal treated with the poisonous portion of proteins, where a subsequent dose of a living germ which certainly would have proved fatal for the normal animal had been given, the symptoms were absolutely identical with those following a nonfatal inoculating dose given to the untreated animal. In other words, to quote Vaughan, "The similarity of the symptoms in the two instances leads us to believe that in all probability we are here dealing with an immunity which is identical in character with that which is usually spoken of as natural immunity." And natural immunity, which by no means could be called specific, is due to a mechanism which is at least intimately connected with the phenomenon of the phagocytosis of the white blood cells.

The tendency today to connect the results of nonspecific protein medication with white cell changes is very evident in recent publications.

2. When we come to consider the behavior of the residue immune animal after inoculation with its homologous living germ the reaction picture indicates an altogether different mechanism. In marked contrast with the normal animal after inoculation, where no toxic symptoms occur under an average of eight hours, the inoculated residue immune animal shows marked symptoms within an hour. After six or eight hours, however, the inoculated treated animal appears in good condition, in comparison with inoculated untreated controls, and eventually recovers, while the latter invariably die of the infection.

In other words, the normal animal and the poison immune animal react identically after the injection of the inoculating dose of virus, and not until after several hours, the former dying of the infection and the latter recovering; while the residue immune, the sensitized, animal, when specifically inoculated, reacts to the inoculating injection in a manner which strongly suggests by its character the reaction following an injection of the protein poison—that is, reacts violently, within an hour, but recovers and is not infected.

Surely these two immunities are not due to the same mechanism!

The most tenable reason for considering the reaction in the sensitized animal to be due to a specific proteolytic mechanism, and the symptoms of intoxication to be due to the liberation of the cell poison of the bacterial protein, is to be found in the fact that, while a reinjection of the cell residue is followed by absolutely no toxic manifestations, the cell residue containing no poison, a reinjection of an equal amount of killed homologous bacterial substance, which contains the poison in combination with the other cellular constituents of the bacterial protein, is followed by symptoms identical with those following an injection of the protein poison.

These unrefuted facts seem to entirely escape the notice of those who criticise Vaughan's theory; while they are met with silence at the hands of most others.

Clinically, the theory of protein sensitization, the development, *not in the blood*, but in the fixed cells of the body, during the course of an infection, or during the course of protein treatment, of a specific ferment capable of the cleavage of the infecting protein, or of one homologous to it, is upheld by many phenomena; among these may be mentioned that of the focal reaction, say, from the injection of tuberculin in the tubercular subject. Experimentally it is upheld, among other phenomena, by that of the response of the plain muscle of the virgin uterus of the sensitized guinea pig, freed from all traces of serum, when touched with a dilute solution of the sensitizing protein, as first demonstrated by Dale.

There is nothing incompatible with this theory, however, in the conception of a cleavage of foreign proteins by the normally present ferments of either the blood or the fixed cells. Indeed, the theory rests upon the ability of the normal ferments to do this very thing; and the specific cleavage action is considered rather an enhanced ability to digest a certain protein than an altogether new function acquired by the body cell—if my interpretation is not a fault. Neither is there anything in the theory which would not include a cleavage of foreign protein by the normally present enzymes of sufficient rapidity, given a sufficient amount of protein, to liberate a toxic dose of its cell poison. This, however,

would be dependent to a great extent upon both the physical state of the protein and the medium of its digestion—its mode of administration.

Thus, the cellular substance of the strain of colon bacillus used in Vaughan's experiments killed when coarsely ground in 1 to 50,000, and when finely ground in 1 to 75,000, or even as high as in 1 to 2,000,000, of the body weight of the untreated experimental animal; while the comparative toxicity of the same toxic protein when given subcutaneously and intravenously, and this includes the crude protein poison, is roughly 1 and 15—that is, such substances are from 10 to 20 times more toxic when given intravenously than when given subcutaneously. Although, as far as eliciting noticeable toxic effects intravenously, rather than fatal effects, is concerned, this may be done with possibly a hundredth part of the dose that causes toxic manifestations subcutaneously.

In nonspecific protein medication, then, we have a characteristic train of symptoms undoubtedly closely connected with the good results obtained, here following in the great majority of instances the most toxic mode of administration of toxic proteins, or of substances capable of precipitating the body proteins; and this train of symptoms is identical with the reaction after a mildly toxic dose of the protein poison given to the experimental animal, as near as it can be duplicated. This identity extends even to the necessity of increasing doses of the protein to produce subsequent reactions, if a subsequent injection is not delayed longer than a few days, or until the tolerance conferred by the poison liberated from the protein of the previous injection has waned. The blood picture is the same; the leukopenia accompanying the rise in temperature, and the leucocytosis, characterized by a relative polymorphonuclear increase, accompanying its fall.

And finally, accompanying, and probably due to, this blood change we have the amelioration of symptoms to a greater or less degree, corresponding to the "temporary immunity," mentioned by Miller, conferred on the experimental animal by treatment with the protein poison. Thus, all evidence seems to indicate that the benefits resulting from the, for the most, intravenous injection of nonspecific proteins are due to the effects of the cell poison liberated by the cleavage of these proteins parenterally, the protein poison of Vaughan.

Although not coming strictly under the head of nonspecific protein medication the work of Gay,<sup>4</sup> who ignores Vaughan's work, is of particular interest here. He treats typhoid fever with intravenous injections of a sensitized polyvalent typhoid vaccine sediment; and, although he apparently assumes his sediment to be nontoxic, in that the "endotoxic substances" are extracted, its exhibition is followed by a reaction in no way differing from those following injection of "unextracted" proteins. In the preparation of this vaccine the multiple strain typhoid bacilli are killed and precipitated with alcohol, and then ground to disintegration; the "endotoxic substances" are then extracted by means of carbolated saline, and the *supernatant fluid rejected*. The sediment remaining only is used.

As a method of detoxication, if this is really the object of the above procedure, a comparison of the effects of the "sediment" of Gay, as evidenced on the typhoid subject, with those of the "cell residue" of Vaughan, obtained by extraction with alkaline alcohol, as evidenced on the guinea pig, will show the



latter to be a far superior method of detoxication, and that, at least in comparison with the cell residue, the sediment is a highly toxic substance.

The cell residue of the typhoid bacillus is absolutely nontoxic for either the unsensitized or sensitized guinea pig in doses of 100 mg. or more when given intraperitoneally—corresponding to about 50 mg. intravenously—and it is very active as a sensitizer. The sediment is toxic for the typhoid patient in doses of 0.02 mg. intravenously; so that, it is very active as a toxic agent at least, and apparently so as a sensitizer.

According to Vaughan, all proteins are toxic parenterally, and *the toxicity of a protein is dependent rather upon the rate of its cleavage than upon its amount*; so that, then, the dose of a vaccine would depend upon two things—the physical state of the protein it contains, and the capability of the recipient for its cleavage parenterally. The fact must not be overlooked, however, that this cleavage may be so rapid and so thorough as to digest the poison itself, converting it into nontoxic bodies. This undoubtedly explains why a sensitized vaccine is less toxic for its specifically sensitized animal than it is for the normal animal<sup>2</sup>—the vaccine, being sensitized, is digested with greater ease, and the animal, being sensitized, digests the homologous protein more rapidly.

Despite his clear demonstration of the beneficial influence of the cell poison upon the resistance to infection in the experimental animal, Vaughan has condemned toxic vaccines. This I consider his one inconsistency. The ultimate test of any theory of immunity, however, must rest with its application clinically. Animal experimentation at best can furnish but a more or less general idea of the effects parenterally of foreign substances upon the human organism, and more especially upon the infected human organism; and this applies with even greater force to the so-called immunity reactions *in vitro*, which take into consideration the defensive mechanism of the blood alone.

Now, if the conception that the parenteral introduction of protein matter may be followed by the activation of the protein poison is correct, then the effects of this poison upon the infected human organism, in contrast with its effect upon the infected experimental animal, are not always deleterious; in fact they are often decidedly beneficial.

The truth of the matter seems to be that the degree of tolerance which different organisms are capable of producing varies, other things being equal, directly with the mass of the organism. The infected laboratory animal has evidently produced tolerance, as a result of the cleavage of the infecting protein, to the full extent of which it is capable; so that, any additional poison, no matter how minute in quantity, or whether given in the form of the cell poison, or in the combined state, as in an homologous protein injection, only hastens the infectious process.

As undoubtedly shown by Gay, the results of the specific treatment of typhoid fever are relatively better than those obtained from its nonspecific treatment. No tenable reason is given for this, however; for the following statements, very evidently made in support of his preference for a specific protein medication, seem to be casual rather than to hold any intended causal significance. These three statements are as follows: (1) The typhoid vaccine "*in addition*" to the benefit derived from its nonspecific reaction "*aids in establishing an active*"

(prophylactic) "immunity against the microorganism concerned." (2) "Re-lapses were distinctly reduced in those cases in which the intravenous injections were followed by a series of three subcutaneous inoculations after the temperature had reached normal." And of prime significance, if a difference in the average reaction picture from that produced in the normal subject is meant (3) "these vaccine injections" of the regular prophylactic dose, 0.1 mg. subcutaneously "in the convalescent typhoid will in a few instances cause a rise in temperature of a degree on the same or following day."<sup>6</sup>

Yet, either of these three statements realize a tenable explanation as to why the specific treatment of typhoid is superior to the nonspecific. This may be summed up in one word *sensitization*; and, in this particular instance, the superior sensitizing qualities of a polyvalent, sensitized, disintegrated typhoid protein over a monovalent, untreated, unground preparation.

With nonautogenous preparations at least, the polyvalency of the vaccine would undoubtedly relatively increase its specificity to the infecting bacterium and, therefore, enhance its *specific* sensitizing qualities as well as its toxicity; the sensitization of the vaccine would probably increase its sensitizing qualities and, as Gay states, its toxicity as well; the disintegration of the bacterial protein would undoubtedly increase both its sensitizing and its toxic qualities, while by the rejection of the supernatant carbolyzed saline solution used in its extraction, both the sensitizing and toxic qualities of the vaccine would undoubtedly be reduced, and reduced, moreover, in direct relation to the fineness of the ground protein, either in suspension or in solution, carried off with the extracting solution. But that there is any appreciable *free* poison either extracted or carried off in this process, from the light of, particularly, Vaughan's work on the extraction of proteins, is extremely improbable.

I have thus, perhaps not too theoretically, discussed the Gay-Claypole Vaccine Sediment for the reason that, although I have had no experience with this preparation, I have been working for a number of years with vaccines which might correctly be called antithetical to this preparation, in two important respects at least. These preparations are unsensitized, and *they may be said to correspond exactly with the supernatant fluid discarded by Gay in the preparation of his vaccine*—all bacterial substance they contain is, at least, ultramicroscopical. On the other hand, these preparations are polyvalent, and unqualifiedly specific in that each one sensitizes, and sensitizes efficiently, only against itself, or against an homologous protein, and sensitizes more readily intravenously than subcutaneously.

From the fact that of these preparations each contains practically an equal amount of bacterial protein in the same state of suspension or solution it has been possible to ascertain that no two of them sensitize with the same facility in the same space of time, or with the same number of treatments, even, apparently, when the same dose of each can be employed, which is not always the case—and that the duration of the state of sensitization against each protein preparation seems to vary inversely to the facility of its production. This duration is a matter of a few months with some of these proteins, and, undoubtedly, of years with at least one other.

These preparations are highly toxic, and being much more toxic intr-

venously than subcutaneously they produce, or enhance, tolerance more quickly when given intravenously; although this can be accomplished quite readily by the subcutaneous method. They produce local reactions when given subcutaneously, but these are diffuse in character and not severe, excepting those following large doses; for they seem invariably to correspond in severity with the constitutional effects from the injection. Given relatively the same degree of sensitization against either of these proteins, and the same dose of each, the character of the reactions, locally and systemically, is exactly the same—that is, one protein seems not a bit more, nor less, toxic than either of the others, produces the same degree of tolerance, and the tolerance following the reaction from one protein is a tolerance to the reaction from either of the others, is of no longer duration, nor differs in any other respect. In contrast with sensitization, this tolerance, by whatever protein produced, if following a single injection has very perceptibly waned by the fourth day; after a series of injections, however, it is of longer duration, but never seems to last longer than two weeks at the most.

Some hundred cases of tuberculosis, among which a few were apparently so acute, many so far advanced and some apparently so hopeless, as to materially affect favorably their amenability to a then not always pleasant mode of treatment, to an extremely rigid regimen and to a monotonous routine of keeping thrice-daily temperature and pulse records, along with other data, furnished the material, in forty-two cases, from the careful study of which the conclusions were definitely settled that *toxic vaccines*, contrary to Vaughan's statements otherwise, are not only the vaccines of choice, but are often actually the vaccines of necessity for the successful treatment of the infections; and that the correctness of his contention that active immunity against a given bacterium, and sensitization against its specific protein, are identical, can be tenably supported clinically.

Every single one of these forty-two cases of tuberculosis gave a more or less frank initial focal reaction to the tuberculoprotein in varying but infinitesimal amount; all of them, with the exception of three, reacted systemically, and about half of them focally, to one or more other bacterial proteins, administered mostly subcutaneously, in varying but much larger doses than were necessary with the tuberculoprotein to elicit the same class of reactions, and two of them reacted systemically to the protein of horse serum.

The tubercle bacillus was found in the sputum, or in other specimens, from the majority of these cases, but not in all; the uncomplicated, early, but well defined, apical and the closed joint cases furnishing most, if not all, of the exceptions. And, although the bacterial flora of well selected sputum or other specimens did act as a certain guide in determining the secondary infectors, the great majority of bacteria here found, and particularly with the sputum, proved apparently to be saprophytic.

On the other hand, neither was the fact that a subject reacted systemically, locally, to a certain bacterial protein taken as conclusive evidence that the homologous bacterium was present as a secondary infector, even when this bacterium was found in a specimen examined. Only the frank focal reaction from a single injection of such a small amount of bacterial protein as to cause



no, or very mild and evanescent, systemic symptoms, and the secondary focal reaction, of tardy onset, following a single injection, or a series of injections, where the reaction systemically was also absent, or very mild but *protracted*, were regarded as specific, and, hence, as evidence that the homologous bacterium was present as a part of a multiple infection.

The so-called secondary focal reaction, that tardily following, as a general thing, the more or less protracted administration of proteins, bacterial or otherwise, and associated with a high and protracted toxicity, can not be regarded as specific, or at least as strictly so. It may be caused by any protein to which the patient will react regardless as to whether it is homologous to the infecting bacterium, or to one of the infecting bacteria of a mixed process, or not. There is no excuse for the administration of vaccines in doses causing such reactions, and the focal manifestations following such systemic reactions should be regarded rather as an exacerbation of the process, than as a reaction. This phenomenon has for its analogy, without doubt, the deleterious effects produced in the infected experimental animal after injections of the protein poison, as demonstrated by Vaughan, and is a nonspecific accident.

As the specific focal reaction, the secondary being practically a protracted form of the primary, will enter largely into the discussion to follow, some consideration as to its probable mechanism, as well as to its significance based thereon, would doubtless be desirable.

Just what determines the predilection of certain proteins for certain organs or tissues is not known; but it is a well recognized fact that not only living pathogenic bacteria infect, show a preference for living, growing and multiplying in, certain tissues, but that these same tissues act as predilection points where these same bacteria deprived of life will be deposited, if injected into the blood stream, and where they are slowly digested. In this manner the cells of these tissues are educated to digest this particular protein, and are then said to be sensitized.

It is easy to conceive of an infected area, in which bacteria are not only living and multiplying, but also dying, becoming highly sensitized in comparison with other, and especially the more distant tissues in less intimate relation with the sensitizing protein; and, if not too completely walled off from the general circulation, of homologous protein introduced parenterally being carried by the blood stream to this sensitized predilection area to be there broken up with the symptoms usual with such cleavage, those of inflammation, in such tissues. These *protein cleavage symptoms* constitute the primary focal reaction; but they are not the only effects from proteins focally. Those from the *protein cleavage products*, or from one of them at least, must receive an equal, if not a greater consideration.

Any attempts to determine the effects of proteins upon the experimental animal by the parenteral introduction of unbroken proteins can only be considered, since the epoch-making studies of Vaughan, on a par with suppositioned efforts to arrive at the definite effects of, say, morphine and apomorphine, by the direct employment of the crude opium from which both are derived. The former procedure is just as irrational as would be the latter, yet it is still being resorted to almost exclusively in immunity studies, both experimentally and



clinically. A doubly flagrant example of this has already been cited in Miller's misquotation of Vaughan's clearly expressed exposition of his well defined work.

In the light of the generally conceded correctness of the observation that an excess of sensitizing protein delays the onset of the sensitized state; of that of the specificity of the refractory condition so clearly demonstrated in the animal sensitized simultaneously to two different proteins, but more especially of the two following demonstrations by Vaughan, it is very evident that the effects of the nontoxic cell residue, the sensitizing portion, of bacterial proteins used as a specific therapeutic agent may not always prove beneficial to the infected subject.

These demonstrations are as follows: 1. It was found that, when injected abdominally into fresh guinea pigs, 25 mg. of colon residue, and 12.5 mg. of typhoid residue, in the short space of thirty minutes, had conferred immunities, in the former case of five, and in the latter of six units; *but that larger doses did not give such good results.* This was tenably explained by supposing that the ferments activated by the residues, for, according to this theory, the ferment is stored in the cell in an inactive state, were in part used up in their reactions with the residues themselves—a relative exhaustion of specific enzyme in each instance, which allows the growth of the inoculated virus.

2. It had been found during two years of treating tuberculosis with the *nontoxic* cell residue of the tubercle bacillus that, if properly used in initial cases, its action was prompt and apparently specifically beneficial; but that in advanced cases it was of no benefit. Of more importance here, however, is the fact that, even in initial cases, *it would prove harmful if given in too large doses, or in small doses too frequently repeated.* The same tenable explanation, it would seem, is equally applicable here.

The tubercular subject possessing systemic sensitization, extrafocal ferment, incapable of the complete cleavage of a large dose, or of smaller too-often-repeated doses, of the tuberculoprotein, here the specific portion of this protein, this specifically active portion is carried to the more highly sensitized predilection point, the focus of the infection, where it uses up the focal ferment in its reaction with this body, with a consequent activity of bacterial life, a more active process, to the detriment of the patient.

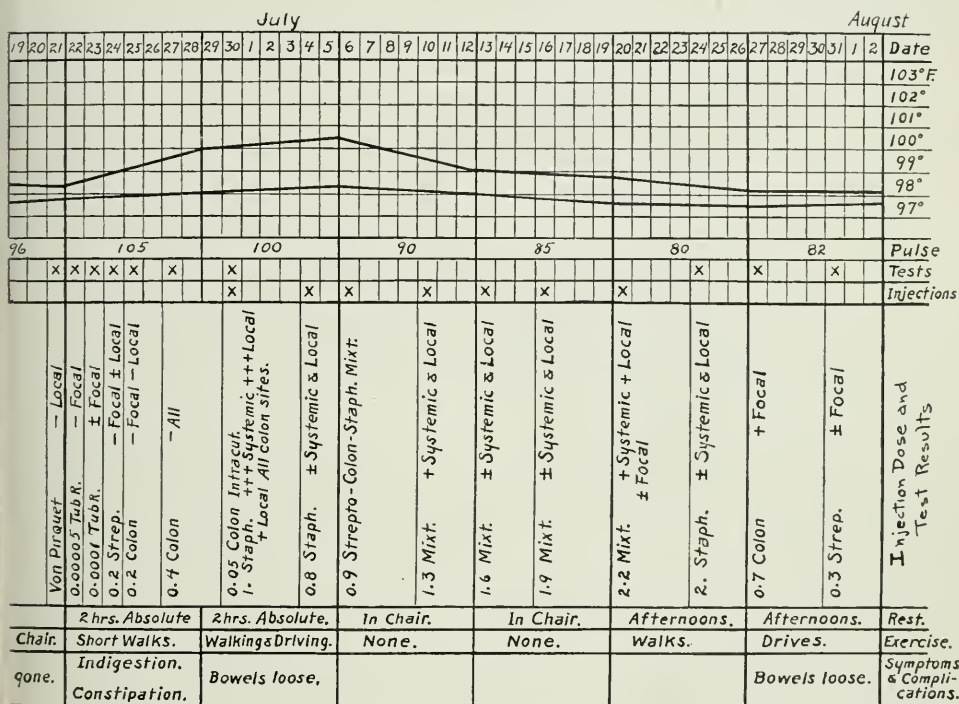
This also explains the secondary focal reaction, which is really an exacerbation of the process of a specific causation; and it is to be noted that its determination from the pseudosecondary focal reaction, previously cited, which is of non-specific causation, the poison, is often made with difficulty when toxic proteins are being employed. This distinction can be fairly well made, however, by noting the character of the systemic reactions immediately following the injections: if these are sharp and evanescent, both the vaccine and its dosage may be said to be beneficial; if they are sluggish and protracted, the secondary focal is indicated, and if they are sharp and protracted, there is the question of an overcome tolerance to be considered—never overlooking the fact that a retrograde of the patient's condition may be due to a double focal cause, both the specific and the nonspecific. Fortunate, indeed, is the tubercular case who gives frank focal responses to proteins used therapeutically; and, by the same token,



This case was that of a man of 27, married, by occupation a mule driver in a coal mine and with a history of exposure to tuberculosis through a sister who had died some time previously of an acute pulmonary process. Another sister, later on, also developed pulmonary tuberculosis; and his wife, still later, strongly evidenced an initial pulmonary infection.

A month or two previous to coming under my care the patient had undergone an ill-advised operation for appendicitis, and subsequently had developed not only an apparently acute pulmonary process, but a multiple submaxillary and cervical adenitis, as well as a diffuse peritonitis. He had been sent home from the hospital to die.

Several of the involved glands had been evacuated while he was in hospital.



and at the time of my first examination it seemed that several more were approaching the stage of dissolution. This, however, did not occur.

He had the appearance, and evidenced the symptoms, both subjective and objective, of an advanced and complicated case of the fulminant pulmonary type, with well defined cavity formation in one lung as the most striking sign of this.

His abdomen was tender and swollen, and he had, and had had for several weeks, an intractable bloody diarrhea. No examination was made of his excreta.

His cough was almost constant, but efficient in that it raised an abundance of purulent, greenish colored, foul-smelling sputum which was tubercle positive, and contained in addition myriads of other bacteria, the streptococcus, staphylococcus and colon bacillus predominating.

His blood pressure was low, his temperature high and his pulse rate rapid

and irregular. Night sweats were constant, and, next to his cough, were the most distressing of his symptoms.

Very little was gained, apparently, from a more rigid regimen; but from a change into more cheerful surroundings and into a larger, more airy, better lighted room, he was certainly better contented.

A week was spent in impressing the patient with the absolute necessity of a strict and protracted adherence to his regimen, in getting him intelligently interested in his case, and in accustoming his mother-nurse to the keeping of an absolutely accurate record. He was then tested for his focal tolerance, his systemic sensitization against, three bacterial proteins—the tuberculo-protein, the streptoprotein and the staphyloprotein. The results of these tests, shown on the case chart, were as follows:

May 10.—A von Pirquet was negative, and so remained.

May 12.—0.00005 c.c. tuberculo-protein, given intracutaneously, produced a well marked focal reaction in both lungs—pain, increased sputum with a little blood—and an increased tenderness over the abdomen. There were no appreciable manifestations of any other kind, and these symptoms subsided during the late afternoon.

May 13.—0.05 c.c. streptoprotein, given subcutaneously, caused no, at least immediately, noticeable response.

May 14.—0.1 c.c. of the same protein caused a very slight redness locally, no marked pulse or temperature variations, but a decided focal response in the lungs, as well as an increased tenderness over the enlarged cervical glands.

May 15.—0.2 c.c. staphyloprotein caused a rise in temperature of 2 degrees, a pulse rate increase of 14, a decided feeling of chilliness followed by sweating and a marked local reaction. No focal manifestations of any kind were noticeable.

This mild but characteristic reaction picture, here following the subcutaneous injection of the protein, was followed the next day by a feeling of decided well-being that continued until the third day, when the temperature began to rise again. From this on injections of the staphyloprotein were given subcutaneously in doses increased each time from 0.1 to 0.3 c.c., and at intervals of from three to five days, until June 10, when 1.4 c.c. was given. This last injection caused a slight rise in temperature and a mild local reaction.

But this reaction picture was not the only thing characteristic of this treatment; besides the well marked amelioration of all untoward symptoms, the variations in temperature and the blood pictures were equally characteristic with the classic reaction picture of this class of tubercular patients *when improving under this treatment*. These will now be considered in their probable order of importance as readily-available clinical guides, or controls for the management of vaccine treated cases.

During the week previous to the tests with the bacterial proteins the mean high, or evening, temperature was 103.7°; and the mean low, or morning temperature 99°—representing a mean difference of 4.7°—and this had practically obtained during a few previous weeks.

During the week these tests were being applied the mean high temperature was 102.7°, and the low 97.3°. A mean difference of 5.4°; a fall in high temperature of 1, and in low of 1.7°.

The week following the last injection of this series, the 1.4 c.c. staphylo-



protein given June 10, shows a mean high temperature of 98.7, and a mean low of 97.7°. A mean difference of 1°; a fall in mean high temperature, which was steady, of 4°, and a rise in mean low temperature, which fluctuated, of 0.4°.

In comparison, it is interesting to note that, four months later, during his treatment with injections of the tuberculoprotein, and after having had practically no reactions of any kind soever for a period of three weeks, his mean temperatures were, high 98.2°, and low 97.66°—a difference of 0.54°. This may be considered as about the normal variation for this case, without doubt. Subsequently, however, when it became necessary to resume other proteins in his further treatment, and systemic reactions were produced, by any protein, including the tuberculoprotein, this variation again increased; but, no matter how severe the reactions, or for what length of time the series of injections lasted, this difference between mean low and high temperatures never exceeded 1.5°, and quickly lessened with each discontinuance of reacting doses of proteins. This is in marked contrast with the initial difference of 4.7°.

That the process of testing the patient to the three bacterial proteins, as described above, had a salutary effect, there can be little doubt; and that this effect was not due wholly, or even to a great extent, to the injection of staphyloprotein on May 15, but in part to the other proteins used previously, is equally obvious. The fall in temperature after this test injection of staphyloprotein was not sufficiently marked to account for the reduced mean temperature occurring during this week; in fact, its tendency was to raise this average.

It must be remembered that the two proteins used in the previous tests were "specific" to his mixed infection; that is, that they caused focal reactions, frankly so. In spite of this, the tuberculoprotein of both the Pirquet and the intracutaneous injection seemed to "flatten out" the high temperature line, and to steadily reduce the low temperature from supernormal to normal, or even subnormal. The behavior of the streptoprotein was somewhat different; for, just as it caused both focal and systemic phenomena, for the local reaction was a mild sign that the latter was really the case, its effects upon temperature seemed to simulate that of the tuberculoprotein upon the low, and that of the staphyloprotein upon the high temperature.

These phenomena could be summed up probably best in the following tentative conclusions. A "certain state of focal activity," whether produced by vaccines capable of producing focal reactions, or by a certain amount of activity on the part of the patient, here including even the change from the horizontal position to the semiprone of sitting propped up in bed, or by a combination of the two, was invariably followed by a flattening of the high temperature line, and by a gradual decline in the low temperature line, which latter quickly tended to reach normal, or subnormal. These manifestations, however, were not of sufficient intensity, or so striking, as to be called reactions. These phenomena must certainly be regarded as beneficial, and of a specific mechanism; though they are incapable of anything but transitory benefit unless produced under the protection of an efficient tolerance.

The phenomena following the administration of the protein not specific to the process, at least in that it caused no focal manifestations, the staphyloprotein, were in marked contrast. Here, these were manifested only after such doses as would cause systemic reactions; and they were characterized, after the re-

action rise, by an immediate and continued effect on the high temperature line, which gradually declined. The effect of these systemic reaction-causing injections on the low temperature line was exactly the opposite. The transitory reaction rise of the high temperature line had its corresponding transitory fall of low temperature the next morning, rarely for two succeeding mornings; but the general, though less regular and steady, trend of this line was from the subnormal to the normal. And, if my interpretations of the observation of many carefully controlled cases are correct, the less regular and less steady trend of this low line from the subnormal to the normal is due to that "certain state of focal activity," which is mentioned above as the probable cause of the gradual decline of this line from the supernormal to the normal, here probably a greater activity.

Among the probable causes of such focal activity, fretting and tossing in bed, both of which this patient did, are of equal importance with those already mentioned. Needless to say, however, these finer temperature variations are only to be noted with any degree of accuracy with the patient at absolute rest, thoroughly under control and carefully watched; and an absolutely essential aid in drawing conclusions is a carefully and properly kept record.

To recapitulate, a certain amount of focal activity, then, undoubtedly makes for a fall in the mean low temperature line from the supernormal to the normal or subnormal, in the early treatment of the advanced tubercular; and this can be considered only as beneficial. A further focal activity, no matter what its cause, tends to keep this line subnormal. A still greater focal activity, probably for the reason that secondary focal phenomena are then manifesting their influence, makes for a rise of this line to again the supernormal mark, just as these so influence the high temperature line also. I shall cite an example of this in connection with testing this case with the colon protein. Needless to say, this rise of average low temperature to the supernormal can be regarded only as deleterious.

I have no explanation to offer as to the exact mechanism of these repeatedly noted phenomena, other than that they are of a specific character, and of focal origin; but as to their significance there can be no doubt. They are considered of sufficient importance to be employed as a certain control in the management of at least the early treated advanced tubercular subject.

During the fourth and last week of this series of staphyloprotein injection the patient was given three tests; a Pirquet was applied the day before the last injection was given, and a subcutaneous injection of 0.00005 c.c. tuberculo-protein was given on June 12, and 0.1 c.c. streptoprotein on June 14. These were the same doses of the same proteins given exactly one month previously; and, as with these previous tests, the Pirquet was negative and both injections caused focal manifestations, although probably less severe than on the former occasion.

The patient was now given a week of rest from active treatment, and during this time he was gradually accustoming himself to spending his days in chair. By this time his night sweats had ceased; blood had disappeared from his stools, and these were now of normal consistency and regularity. In addition, his cough was no longer troublesome, his sputum had become much reduced in quantity and was now only streakedly yellow; it had lost its foul od-

and contained a very perceptibly reduced number of bacteria, especially of the streptococci. Moreover he had gained materially in weight and appearance.

At the end of this week of rest his temperature was again on the upgrade. A Pirquet was applied, again with negative results; but he now tolerated 0.0001 c.c. tuberculo-protein with but faint focal manifestations, and 0.2 c.c. streptoprotein without the slightest signs of any such phenomena. This latter protein, however, did cause a possible slight rise in temperature with a mild local reaction.

About two weeks, then, after the last of nine subcutaneous injections of staphyloprotein, extending over a period of four weeks, during which his sensitization against this protein increased about 120,000 units, the patient had gained in sensitization against the tuberculo-protein to the extent of about five units, and against the streptoprotein about 10,000 units. And here be it understood that the word "sensitization" is not strictly applicable in describing the increased "tolerance" of the patient to the staphyloprotein. This was undoubtedly due in great part to increased sensitization, but to some extent also to the tolerance of the poison he had acquired through his previous injections. That this tolerance existed at the time of the last injection of the series, 1.4 c.c., which caused a mild reaction, and that it had practically disappeared twenty days later, was evidenced by the overwhelming reaction following 1 c.c. of the same protein then given. The focal reaction, as evincing the degree of sensitization, would probably have been absent with any safe dose of this protein.

Why this gain in sensitization against the tuberculo- and streptoproteins was not evident immediately after the last injection of the series of staphyloprotein injections can not possibly be explained by the influence of tolerance, as can be readily seen; but it might be explained here, and in similar cases, and very tenably so, by supposing that the body cells no longer being subjected to the influence of the more readily sensitizing staphyloprotein of the injections were now being stimulated by the less readily sensitizing autovaccines of the tuberculo- and streptoproteins of the infecting bacteria to the end that a further sensitization against these bacteria was now being produced. This would resume that the autostaphyloprotein had been cleared up by its specific treatment; and, indeed, evidence of this existed in the almost total absence of staphylococci from the sputum, if not in the general improvement of the patient. Evidence of this increase in sensitization, however, can at times be elicited immediately after such a course of vaccines.

In testing with the colon protein, at the expiration of his rest period, it was found that neither 0.2 c.c. nor apparently 0.4 c.c. of this protein caused the slightest reaction of any kind; so that, some doubt existed as to whether the colon bacillus was responsible in part for the mixed process, or, as would naturally follow, whether he was sensitized to this protein.

The high temperature line alone seemed to be affected by these two injections; but, as the patient had been increasing his activities a little too rapidly, and was living more quietly at this time, it was a matter of doubt as to whether the gradual fall in temperature which then occurred, a matter of a degree, was due to this rather than to the vaccine. To ascertain which was the real cause an intracutaneous injection of 0.05 c.c. colon protein was given on June 30, and



this was followed immediately by a subcutaneous injection of 1 c.c. staphyloprotein.

The severity of the reaction caused by this injection has already been noted. The temperature rose 5°, and there was a decided chill followed by sweating, as well as a very severe local reaction. The site of the intracutaneous colon protein injection, on an entirely different part of the body, and not near the sites of the previous subcutaneous injections of this protein, lighted up and showed distinctly positive, as did the sites of the two subcutaneous injections also. This phenomenon will be discussed a little later.

Theoretically this dose of staphyloprotein was correct; practically it was too large by far; for no account was taken of the different conditions under which the patient was then living in comparison with the time when 1.4 c.c. of this protein had caused only a moderate reaction. He was decidedly more active, and partly as a result of this autointoxication had already overcome the tolerance then existing, for he was evidencing a gradually rising temperature. And, as it is the liberated poison above tolerance which causes the reaction, he practically got the full effects of a very toxic cleavage of the total dose; for his sensitization against this protein was then, without any doubt, high.

Very little significance was placed at the time in a decided looseness of the bowels which set in after this reaction. This was attributed, as was the four day rise in temperature, *both morning and evening*, although the latter to a much greater extent, naturally, to the severe reaction. This then seemed a correct conclusion as these untoward symptoms quickly subsided after another injection, now of 0.8 c.c. of staphyloprotein on July 4, which caused a mild systemic and local reaction. Later, however, undisputable evidence that this was a focal manifestation, *secondary in character*, possibly caused by the colon protein of the two injections, and probably aided and abetted by the severe reaction, was found, and in this manner.

He was now given a little over two weeks of treatment with a mixed vaccine composed of one part strepto-, two parts colon and four parts staphyloprotein, to all of which he had reacted. This series of five injections started with 0.9 c.c. and ended with 2.2 c.c. of this mixture. The last dose caused not only a mild systemic response, but focal manifestations as well—frankly, but a decided abdominal tenderness, questionably, by an uncomfortable feeling over the chest. The only other sign of this was a slight rise in mean morning temperature—but, as already stated, with even a moderately active patient not much dependence can be placed in these delicate variations when they occur alone. Here, however, this questionable additional evidence of focal activity was not needed; for some reason he now frankly evidenced focally a too large dose of one, or more, of the proteins of the mixture.

The question now was which of the three bacterial proteins had caused these focal reactions. To this end, and although two alone were suspected, subcutaneous injection of 2 c.c. staphyloprotein was given four days later, July 24. This caused only a mild response locally. On July 27 0.7 c.c. colon protein elicited a frank focal response in the form of a slight abdominal tenderness and a slight tendency to diarrhea, no temperature variations were noticeable. On July 31 0.3 c.c. streptoprotein caused a mild tenderness of the cervical glands.



and a decided feeling of oppression over the chest, but no temperature variations.

Undoubtedly two of the proteins, the colon and the strepto, were in part the cause of the focal manifestations following the mixed protein injections; while the temperature excursion, partly at least, and the diarrhea following the tests with the colon protein were undoubtedly due to a mild exacerbation, a secondary focal reaction, probably brought on by, if not directly due to, the two injections of the colon protein given a few days before, and from which no reactions of any kind were noticed.

Four weeks were now devoted to careful tests with the different proteins, the while the patient observed a strict regimen, especially as to exercise and rest. The results of some of these tests, those most pertinent to the question, with their discussion, follow.

A Pirquet was again applied, Aug. 21, and this remaining negative, a subcutaneous injection of 0.9 c.c. staphyloprotein was given two days later. This was followed by a reasonably severe systemic reaction with local manifestations, while the Pirquet lighted up and showed strongly positive.

It is a well recognized fact that, other things being equal, the tubercular subject who shows a frank local response to the tuberculoprotein, whether exhibited in the form of a Pirquet or an intracutaneous, or subcutaneous, injection being immaterial, as the mechanism is the same in each instance, is the subject who, as a general thing, responds the most readily to treatment. This is undoubtedly due to the fact that such a subject possesses a relatively high degree of sensitization against the tuberculoprotein. But that there are some other cases not so reacting to the tuberculoprotein who respond equally well to treatment is equally true; and this case was one among this number.

Despite the evidence that the local reaction is determined by the *cleavage* of proteins, and not by the *products* of their cleavage, it is very evident that tolerance, *produced by a protein cleavage product*, does have a certain effect upon the local reaction.

It had been noted times without number that a Pirquet, or the site of an intracutaneous, or of a subcutaneous, injection of tuberculoprotein, showing negative, would often light up and show positive after the injection of another, or of the same, protein given in such dose as to cause a systemic reaction; in other words, by overcoming the then existing tolerance. This nonspecific, and specific, lighting up of injection sites had been noted with other proteins as well, and was demonstrated previously in this case, nonspecifically, by the lighting up of the intracutaneous site of the colon protein injection with the systemic reaction following the subcutaneous injection of staphyloprotein, already cited, and it was really demonstrated specifically by every local reaction that occurred.

The fact that the sites of the subcutaneous injections of colon protein also lighted up with this supplemental injection of staphyloprotein, since one had been given four, and the other six, days previously, should have indicated what was the probable reason for the absence of reactions systemically, to the extent at least of preventing the further employment of this protein, and especially in mixture. Had this occurred a little earlier in the treatment, the results might well have proved disastrous.

The negative Pirquets, and the negative intracutaneous injection of tuberculo-protein, in this case were interpreted, especially after their failure to light up with the reactions of supplemental injections, as showing that the patient possessed insufficient extrafocal, or systemic, sensitization against the tuberculo-protein to break this protein up with sufficient violence to cause local manifestations of inflammation—and proteins, the tuberculo-protein in particular, are broken up possibly the most slowly in the skin, just as they are probably broken up the most rapidly in the blood stream.

And the same interpretation, somewhat modified, should have been made in the case of the colon protein—a temporary exhaustion of its specific ferment, due possibly to digestive disturbances, which the patient really had at that time. So that, the colon protein, instead of being beneficial, as would be judged by the fact of its producing no reactions, while the temperature seemed to be affected favorably, was really doing harm. I have no explanation as to why this protein failed to produce focal manifestations frankly in this particular instance, and then did so later; and especially is this puzzling as the frank focal manifestations occurred after a mixed vaccine, and not with the straight. My experience has been to expect just the opposite, which is one reason that I consider mixed vaccines dangerous.

This, the fourth application of the Pirquet showed positive, but only after a supplementary injection which produced a systemic reaction. An unsupplemented local reaction from the tuberculo-protein was not noticed with this particular patient until the dose of this protein had reached 0.4 c.c.—and, it may be added, that he had then had no injections of other proteins for close on to seven weeks, and was living under a careful regimen; so that it required very little in the way of a dose of protein to overcome what tolerance then existed.

Special care was always exercised in giving the tuberculo-protein injection to have a certain amount deposited within the skin, as well as to avoid previous injection sites; so that, with the largest dose reached with this protein, and after a few repetitions of this dose had ceased to show a reaction of any kind, it was superfluous to apply a Pirquet, or to give an intracutaneous injection of the protein to furnish additional evidence that the protein both in the skin and under the skin was being digested so readily and so thoroughly as to cause no symptoms either from its cleavage or from its cleavage product.

It is of significance to note that, over one year after the patient had received his last tuberculo-protein injection, an intracutaneous injection of 0.05 c.c. of this protein, even with a supplemental injection of a strepto-mixed vaccine, which he was still partially sensitized, and which caused a rather sharp systemic response, absolutely failed to light up. So that, the Pirquet, or its analogies, irrespective of the influence of tolerance, will show negative, then positive, and again negative, as *sensitization advances*.

That this was undoubtedly happening with this subject there is other abundant evidence. Part of this evidence is shown by the results of the other tests made at the time of the positive Pirquet. On Aug. 25 he was found to tolerate 0.5 c.c. streptoprotein, and on Aug. 30 0.0008 c.c. tuberculo-protein was tolerated equally as well; while a little previous to the application of the po-

tive Pirquet he was found to tolerate 0.7 c.c. colon protein, which then gave a systemic response.

I have already shown the increase in sensitization against the tuberculo-protein during the first series of injections, four weeks of treatment with the staphyloprotein, to have been about five units; while now, after seven weeks of regimen and testing, including the second series of injections, three weeks of staphylo-colon-streptoprotein treatment, his gain in sensitization against the tuberculo-protein is more than 70 units.

This obvious increase in gain of sensitization during the second series of injections over that obtained during the first series, absolutely does not evince any superiority of the mixed vaccine over one of a single kind of bacterial protein, a *straight* vaccine, such as was the streptoprotein used during the first series of injections; but, in my opinion, which is based on controlled observations of many cases treated with both straight and mixed vaccines, including autogenous preparations of both kinds, this more rapid increase in sensitization is due to the increased ability of the patient to combat his infection. If anything, the mixed vaccine is more or less of a handicap, depending upon the number of different kinds of bacterial proteins entering into the mixture, and, by the same token, upon the multiplicity of the mixed infection against which it is employed.

However superior to straight vaccines for the production of the nonspecific tolerance in selected cases, the mixed vaccines are unquestionably less efficient as "specific" sensitizers, and are more dangerous under all circumstances in mixed infections. This is as should be expected, if the matter of the production of bacterial immunity is regarded rationally, from the point of view of the physiologic effects of remedial agents, here, for all practical purposes, two in number—the toxic portion and the nontoxic portion of the protein molecule—and is not viewed as the effect of a single agent, and more especially when "camouflaged" as *antibodies* produced by an *antigen*.

The gain in sensitization against the streptoprotein during the first series of injections was, as already stated, 10,000 units. This gain during the second series was over 40,000 units—much less comparatively than the gain against the tuberculo-protein during the same period and with the same treatment, but not under the same conditions. This gain, actually, should have been more, not only on account of the improved condition of the patient, and for the reason that the bacterial protein of this microorganism is a better sensitizer than that of the tubercle bacillus, but principally because the strepto infection was treated *specifically* during the second series of injections, the mixed vaccine containing streptoprotein. This was not the case with the vaccine used in the first series of injections.

Other than stating that the streptoprotein has always proved a "mean" protein with which to work, and that streptococcic infections, at least when secondary to a concomitant tubercular infection in its early management, seem to be more safely and more satisfactorily treated by a nonspecific therapy, the only further explanation I shall offer for this failure of a greater increase in sensitization against this protein is that the focal phenomena, finally noticed with the mixed vaccine, acted as an inhibiting factor.

This was undoubtedly the case with the colon protein, against which the



initial degree of sensitization was not ascertained. The three weeks of colon protein injections of the mixed vaccine, however, increased sensitization against this protein about 30,000 units, which was a very small increase for the number of injections employed.

I am strengthened in my opinion that the focal phenomena are the principal factors here, not only from my observations of the sensitizing qualities of these two proteins with many other cases, but from their later behavior in this case. A very little later in his treatment this patient evidenced a focal tolerance of the colon protein, after only a few injections of this protein used as a straight vaccine, which equaled 200,000 units; while still a little later a focal tolerance was obtained with a few injections of the streptoprotein of 120,000 units. All of these injections, although causing systemic reactions, were devoid of any effects focally; but it is possible that their respective bacteria had then ceased to be focal factors, that, by this time, the mixed process had been converted into an unmixed tubercular infection.

This tenable conclusion would seem to be upheld by *the rapid improvement of this patient noted after the focal manifestations produced by the colon and streptoproteins had subsided*. This phenomenon has been often noted in other cases, after the initial stage of improvement, and with other bacterial proteins, including the tuberculoprotein particularly in infections of the joints.

With this case it was also ascertained that sensitization against the staphyloprotein had very perceptibly lessened at the end of twelve weeks; and that, with the colon protein, this was not the case at the end of eleven weeks. At the end of twelve months sensitization against the streptoprotein, although it had waned perceptibly, was still efficient for the production of a sharp reaction after an injection with that protein, which indicated it had lost nearly one-half. In this respect the tuberculoprotein is apparently in a class of its own; for, at the expiration of one year and six months, absolutely no evidence was apparent with this case that his sensitization against this protein was not as high as it had been at the conclusion of his treatment.

It required eighty-four weeks to sensitize this subject to the tuberculoprotein sufficiently for 2 c.c. not to cause a systemic reaction when given subcutaneously thirty-two weeks to sensitize him against the streptoprotein so that 1.2 c.c. caused but mild systemic symptoms; twenty weeks for like but more toxic results from 2 c.c. colon protein—and the sensitizations against the two latter, and especially the colon protein, were undoubtedly delayed by untoward focal phenomena—while an equal degree of sensitization to that against the tuberculoprotein, the poorest sensitizing protein of them all, could have been obtained against the staphyloprotein, the best sensitizer, in probably six weeks at most.

The above relation as sensitizers, and as evincing the comparative virulence of the above-named bacterial proteins has proved practically the same in every single one of some hundred cases where one or more of these bacteria have been found as concomitant infectors with the tubercle bacillus; and this fact alone furnishes the most potent of reasons against the employment of mixed vaccines that can possibly be advanced. This is not a haphazard conclusion, nor is it based alone on the behavior of these four different bacterial proteins upon the infected subject; for, although these differences are accentuated possibly



with the multiple infected, they absolutely obtain to a possibly less extent with the uninfected. Almost absolutely exact comparisons were possible with these protein preparations for the reason, as already given, that they each contain a like amount of their respective bacterial substance, of a multiple strain, to each cubic centimeter, of a like cellular fineness in suspension or solution.

As a sensitizer the polyvalent colon protein stands between the staphylo- and streptoprotein preparations, but much nearer the former than the latter. It is a good protein with which to work. As to the polyvalent streptoprotein, as has been said, it has proved so unexpectedly puzzling with which to work at times, that, in the more acute cases especially, if this protein is found to be specific to a concomitant infector, and the case is found not to react to some other, and less "aggressive," protein, it has been, and still is, my custom to deliberately sensitize against, preferably, the staphyloprotein, and employ this protein to control tolerance.

This process of sensitizing the subject to this protein preparation can be accomplished generally after three intravenous injections, followed by a wait of two weeks, when the former nontoxic initial sensitizing dose almost invariably causes the classical reaction when employed intravenously. This initial dose averages 0.1 c.c. After a few additional intravenous injections the patient will invariably begin to react to, of course, a much larger dose given subcutaneously. It may be added irrelevantly that this preparation, as well as the colon protein, has been doing yeoman service in the tropics for two years in the treatment, nonspecifically, of certain chronic skin affections, and of the so-called tropical ulcer, many of them of undiscovered etiology.

Additional evidence that this patient was being gradually and efficiently sensitized to the tuberculoprotein is found in the following two experiments. It had been found quite accidentally that when a subject would tolerate a certain dose of any of these bacterial protein preparations when given subcutaneously, and when this systemic tolerance of the protein was mostly due to sensitization, and not to a tolerance of the poison, that from 1/100 to 1/20 of that dose of the same protein could be given intravenously with safety, although it was followed by a chill. When this patient could tolerate 1 c.c. tuberculoprotein in the manner mentioned, and after a sufficient time had elapsed to allow any possible tolerance to wane, 0.025 c.c., or 1/40 of the nontoxic subcutaneous dose, was given intravenously. This was followed in a half-hour by a chill, and the classical reaction, in no way differing from that caused by any other protein. His degree of sensitization at that time was not sufficient to break up 0.025 c.c. of his particular protein beyond the toxic stage of its cleavage intravenously.

When, however, the patient could tolerate 2 c.c. of this protein subcutaneously this intravenous procedure was repeated, but with absolutely not the slightest noticeable reaction of any kind soever. Very obviously 0.025 c.c. tuberculoprotein could now be broken up beyond its toxic state of cleavage when introduced into the blood stream.

This exposition must not be considered as a model for the management, or even treatment, of the tubercular process, but rather as a skeleton upon which a model for each class of this infection of such varied manifestations might be partly constructed and finally built to fit the exigencies of each particular case.

This work was all completed over three years since, and has been reviewed and studied, written and rewritten, and compared with much of the more recent work, especially with that having to do with the nonspecific treatment, with proteins, of, for the most, chronic infections. I have attempted to reconcile my results with other conceptions of immunity at more or less variance with Vaughan's theory of protein sensitization, notably those of Jobling and Peterson, and Bronfenbrenner; but with results not complimentary to these other conceptions, mostly in that they are not reconcilable with two fundamentally important considerations—the patient and the remedial agent. The doubly, or multiply, infected organism finds no place in these schemes; and the double physiological effect of proteins is entirely overlooked.

If there are any flaws in the interpretations of the results herein set forth, which, with the exception of certain views on toxicity, are but the clinical application of Vaughan's experimental conclusions, a great deal of additional work with vaccines upon a variety of other infections done since has, so far, failed to make these evident. However, of this I am certain, of the two factors entering into the successful treatment of the infections, in so far as protein therapy alone is concerned, the nonspecific and the specific, the former is of the more importance, in that it paves the way, so to speak, for the more delicate procedure of specific therapy, *when this is necessary*; and with tuberculosis, for the consummation of the much more efficient, much more enduring, results of this therapy, a hypersensitization against the most aggressive infector of, in the great majority of instances, a multiple process, the tubercle bacillus.

I have been repeatedly asked why, when such good results so often follow a nonspecific therapy in tubercular infections, run the risks of the more dangerous administration of the homologous protein. The answer differs only in one respect from one of Gay's presumable reasons for preferring a specific to a nonspecific therapy in typhoid, which was that "*in addition* it will produce an active immunity." My answer always has been that the tuberculo-protein *only* will produce an active immunity; and it might be added that after the good results have been obtained from a nonspecific therapy, the specific therapy is no longer any more dangerous than the nonspecific. That it is the more efficient of the two, note the following:

After 24 weeks of treatment with proteins other than the tuberculo-protein, this patient had gained in sensitization against the latter about 6,000 units; but after only seven weeks of treatment with the tuberculo-protein his gain was very much over 44,000 units.

If there was any reason for having continued the tuberculo-protein in this case until 2 c.c. caused no perceptible reaction it was because it had required on an average of 2 c.c. of other proteins to control his tolerance at various times. This dose would represent 200,000 units; but how much beyond this figure his degree of sensitization really had reached I do not know; although, without much doubt, it was higher.

In the first place, from the time he was given 1 c.c., when he was receiving his injections once a week, only ten additional treatment injections of the tuberculo-protein were used, first at two-week intervals, and then at intervals of a month. From none of these injections was there ever any but a very mild

local reaction, and not always this; so that these might well have been due solely to some of the injected protein being deposited in areas of skin previously locally sensitized by one or more of his now many injections of this protein.

In the second place, eighteen months showed no abatement of his sensitization from a repetition of this dose; and, as stated this was verified by an intracutaneous injection of tuberculo-protein supplemented with a subcutaneous injection of a protein to which he did react, as well as by an intravenous injection of a former nontoxic dose of this protein. So that, rather than evincing an undiminished sensitization, these tests really demonstrated a sensitization not diminished to such an extent as to cause a toxic cleavage of a former nontoxic dose of the tuberculo-protein.

Two years after his discharge as cured, long before which time he had resumed his former occupation—in fact, he had done this several months before his discharge—this former pitiable object of humanity was the picture of perfect health, and, with the exception of a very perceptibly retracted right chest wall, and some healthy scars of the neck, bore absolutely no signs of his severe infection. Three years after, inquiries from an interested friend elicited the information that he was still a hard working, perfectly healthy man, with habits of life in marked contrast, for the better, with those of his pre-treatment days—and this has practically been the later histories of some twenty others, in so far as it has been possible to learn of these former advanced tubercular patients.

As to the blood, this case was unfortunately not among the number upon which counts were, at least regularly, made; nor, as will be seen, are the conclusions following based upon the actual number of cases where counts were made, so much as upon comparative counts made during the absence, with those made during the presence, of that nonspecific condition here designated as tolerance. The following tables are really based upon counts from 12 cases, 36 in number, and so divided that the two extremes, and the mean, changes in the small lymphocytes will show a fair average; and it is, let it be added, only very recently that these counts have been arranged for study, and certain deductions drawn therefrom, although they were all made several years since for a somewhat different purpose.

This purpose may be said to have had its inception in the observation of Friedberger and Schymanowski, cited by Vaughan,<sup>7</sup> that the presence of leukocytes seems to prevent the formation of *anaphylatoxin*, which, whatever its real matrix, is a *protein poison*, and that they apparently destroy it when abundantly formed. The suggestion of Vaughan that the leucocytes digest the poison and convert it into a harmless body, taken in conjunction with the noted leucocytosis early following the injection of proteins, seemed to offer a possible means of controlling injections given for the purpose of producing tolerance, and of maintaining it at efficiency; that is, of determining the intervals between treatment injections.

This opinion, as to the utility of the numerical white count, has now been broadened to the extent that the *blood picture* is considered of equal, if not greater, importance than the differential white count, and that this differential, if the smears are carefully and uniformly prepared, will show all that, or more than, will the numerical count, even to the extent of obviating the possible neces-



sity of making the latter; and, in that this picture offers a means of no mean order for treatment control, in that it probably offers very early evidence, not only of an ill-directed therapy, which is very desirable, but also of the progress of the patient at an earlier date than any other signs indicate. As to its offering any sure means of gauging injection intervals I am still somewhat in doubt.

First as to the blood picture, strictly speaking. Possibly due to the fact that my cases of tuberculosis have been, for the most, advanced ones, I was early struck by the difference in appearance of the white cells of some of the smears made for differential counting, and, in those few instances where they were made, by a certain difficulty in making numerical counts of blood from the same patients, when compared with other specimens. At that time this was at first thought to be due to faulty preparation of specimens, in that most of them were carried from the bedside to the laboratory for fixing, staining and examination. No account was taken of the fact that my office cases were mostly far along in convalescence, and furnished the good specimens, and that my bed-ridden cases were of particularly ugly types, and furnished the bad specimens; so that, this difference between the two classes was attributed to a possible drying out of the specimens before fixing.

The fixing of specimens at the bedside was easy of accomplishment; but this offered no solution. Before the institution of a portable microscope and staining outfit were well established, however, some of these specimens began to assume a different appearance. The white cells from those patients who were improving the more satisfactorily, or rather, from those who did so later on, were gradually assuming a fatter, more rotund, healthier appearance; were taking the stain better, could be more readily differentiated and, quite apart from their transitory increase following each injection, with the accompanying relative neutrophile increase, they were assuming an altered relation differentially in other respects, if really not a more gradual, more lasting, although less marked increase numerically.

Counting the large mononuclears and the transitionals as large lymphocytes and the eosinophiles and mast cells as polymorphonuclears, for the reason that this procedure, now recognized as at least unfortunate, greatly facilitated the making of many and repeated counts, the following table is submitted as showing the normal percentage of white cells according to this division; the approximate average of change from the normal with the advanced tuberculous process having one or more secondary infectors, and the effects, a more or less prompt alteration of this latter, produced by the injection of one or more beneficial doses of protein—that is, the average differential picture of the mixed tubercular subject with an inefficient tolerance, and that of the same patient after a more or less efficient tolerance has been established, in comparison with the normal picture.

The counts, the average results of which are portrayed in the third line of the table, it must be understood, were invariably made from two days to one week after any injection of the treatment protein; that is, after the transient leucocytosis immediately following the injection had subsided. For the purpose of making these counts, as has been said, was merely to regulate protein treatments.



	SMALL LYMPHS	LARGE LYMPHS	POLYMORPHS
Normal	22%	11%	67%
Inefficient tolerance	20%	16%	64%
Efficient tolerance	2%	24%	74%

From this table it can be seen that the low small lymphocyte count, invariably associated with correspondingly high large lymphocyte and neutrophile counts, may be said to constitute the most prominent characteristic picture of the early improving patient; and, when this occurs, or even when a tendency towards this changed numerical arrangement obtains and is accompanied by the improved physical appearance, if the term be permitted, of the cells, it can be regarded as infallible evidence that the effects of the protein injections are beneficial to the patient.

As to the promptness with which the maximum change in the small lymphocyte may occur, note the following: Among the half-dozen cases where zero was reached with respect to this cell, this was noted in one joint case with an initial 17 per cent after three injections given at three and four day intervals; while, in marked contrast, with another case, an ambulant pulmonary with an initial 24 per cent, this occurred only after thirty weeks of treatment, many protein injections, and after the ninth injection of the tuberculo-protein.

On the other hand, the highest low percentage of small lymphocytes noticed in a bed-ridden pulmonary, 16 per cent from an initial 24 per cent, was obtained only after the eighteenth injection of a protein other than the tuberculo-protein; while with another pulmonary, also under a strict regimen, a reduction from the initial 28 per cent to 9 per cent was noted after a single injection of a protein homologous to a secondary infecter.

It is interesting as well as significant to note that with still another advanced pulmonary an excursion of temperature following an attack of indigestion, *not in exacerbation of the process*, be it understood, acted more efficiently than a dose of vaccine, or even than a large number of them, in that it further reduced an already reduced minimum 10 per cent to zero—probably the effect of an absolutely specific autovaccine "activated" by the intestinal irritation accompanying the indigestion. This case reacted focally to the colon protein, be it noted.

All of these cases had shown a tendency, more or less marked, towards the low small lymphocyte count from the very first, or first few injections of vaccines.

There seems to be nothing to indicate any specific action in this, other than that, while a return towards the differential average of "inefficient tolerance" seems to be immediate after too large or too frequently repeated injections of proteins which cause systemic reactions, but no focal manifestations, this is only less immediate after injections of vaccines which do cause focal manifestations in that the influence of these manifestations, which are here secondary specific phenomena, is not exerted as promptly after the injection as in the former case. In other words, the same focal phenomena making for the greater variation between morning and evening temperatures, already discussed, seems equally to make for the undoubtedly less satisfactory blood picture, including the partial differential of the second line of the above table. To what practical application this could be put remains to be worked out.

Let us consider this question from another viewpoint, at least in so far as the above division of white cells will permit, viz.:

	LYMPHOCYTES	LEUCOCYTES
Normal	33%	67%
Inefficient tolerance	36%	64%
Efficient tolerance	26%	74%

This table would seem to place the weight of evidence greatly in favor of the leucocytes as being at least the principal factors responsible for the production of the nonspecific beneficial condition of the infected organism here designated as tolerance.

Again, considering the above table from the point of view of the phagocytic action of the white cells, the following deductions are both interesting and instructive. The lymphocytes, if not constituting the *macrophages* of Metchnikoff, may be said to include these phagocytes, the large mononuclears. One function of these cells is certainly the digestion of the metabolic detritus of the organism. The leucocytes in reality are the *microphages* of this illustrious savant; and it is these cells that play the important role in bacterial infections.

Now, at the time when the destructive changes in the cells of the infected organism, and these would necessarily include the leucocytes, would naturally be considered the highest, during an active bacterial process, when, in other words, *tolerance is inefficient* the lymphocyte percentage is at its high mark and the leucocyte at its low; while, at the time that these destructive changes, if not at their lowest, are at least less high, during a rest in, or a retrograde of bacterial activity, *when tolerance is efficient*, the lymphocyte is at its lowest, and the leucocyte at its highest mark.

The exact figures are here immaterial, for they are necessarily of the most relative, and beside the point; it is the facts that count, and these are too obvious to be ignored.

There are other and quite tenable deductions to be drawn from these partial differential counts; but, lacking the percentages of especially the large mononuclears and the transitionals, as well as accurate simultaneous numerical counts which were made on insufficiently numerous occasions, these would be open to criticism as being premature conclusions.

However, it may be said that in every instance where a numerical count was made in conjunction with either the above partial differential, or with the differential of wider scope, there was invariably a greater or lesser increase above the normal of the total number of white cells with the intolerant cases and as invariably an increase of the same kind, perhaps uniformly higher, with the tolerant subjects—in no instance, however, nearly equaling the transitory increase early following the protein injection.

Further, it may be said that the increase in the intolerant cases seemed to bear a direct relation to the multiplicity of the mixed infection; while the increase in the tolerant subject undoubtedly bore a direct relation to *both the sharpness and the evanescence* of the reactions produced by the protein injections, which undoubtedly indicates a higher relative specificity of the protein employed.

The tendency of this composite blood picture, however, was to early and quickly revert to the normal with the consummation of an efficient tolerance and the discontinuance of reaction producing doses of vaccine, and again to return to the "inefficient tolerance" picture if tolerance became inadequate, for any reason, or focal reaction producing injections were given. And this could and did occur a number of times with many cases, irrespective of the stage of the treatment—in other words, *this blood picture had absolutely nothing to do with the degree of sensitization acquired by the patient*; while the degree of sensitization, as already shown, had everything to do with the permanent cure, or the specific immunity, established.

The tubercular patient with a semiacute process and a readily demonstrated lesion, whether in the form of a more or less circumscribed pulmonary, focus or another similar lesion, for obvious reasons is the ideal subject for the study of protein therapy, and of its effects in relation with the progress of the infection. Such a subject in which the symptoms of the malady are visualized as the primary effects upon the infected organism of the parenteral cleavage of bacterial proteins of an absolute specificity, and secondarily so through the effects of this cleavage upon the infecting microorganisms, or on the process, if it is considered as a living, increasing or active, or, decreasing or inactive, entity, is far superior to any test tube efforts aimed at such a study.

The symptoms of a protein therapy, both the reactions and the results, although necessarily regarded in exactly the same light, must in addition be viewed as the effects of probably relatively less specific proteins, but of proteins whose potential qualities have been enhanced by modifying them physically to a greater or less extent; that is, in that they have been rendered capable of a more ready cleavage and, up to a certain point in their cleavage, of a greater toxicity, as well as capable of more readily sensitizing the subject against the homologous protein of the bacterial invader.

There is absolutely no other construction to be put on the change in the proteins of the preparations so far successfully employed in vaccination—from the time of the first authentic employment by Jenner of the relatively less specific, less virulent, virus of cowpox in prophylactically vaccinating against smallpox, to the modification of the virulence of the pathogenic virus by animal passage, as practiced by Pasteur, down to the more recent "sensitization" of the bacteria, as first practiced by Besredka, and the disintegration of the bacterial cells by mechanical means—as tenable as that this change is due to the fact that the virus, or the bacterial cellular substance, so employed is, or has been rendered, less resistant than the infecting virus or pathogenic bacteria.

Whatever the ostensible purpose of the method of modifying the bacterial protein for therapeutic application, and this has been various, this one absolutely essential thing has been accomplished, whether the original one has or not— invariably the protein has been made more susceptible to cleavage by the parenteral enzymes; and the only exception as to its toxicity having been at the same time relatively increased, is to be found in the case of Vaughan's "cell residues," from which the poison, an integral part of the protein, and not a re-formed toxic body, has been removed by disrupting the protein molecule chemically and extracting it with absolute alcohol, in which the poison is soluble.



As to the proper designation of this therapeutic agent, the following is submitted. As toxins and ferments, both undoubtedly substances of a like, or enzymotic, nature, are correctly included among the antigens, if, indeed, they do not wholly constitute this class of hypothetical bodies, then the sensitizing proteins *per se* may not be rationally so included, since the bodies formed against these substances, in the light of Vaughan's still unrefuted advancements, are themselves enzymotic in nature, and, unlike the antibodies, are not formed in the blood. Neither can the bodies formed against the sensitizing proteins be rationally termed antibodies, which are hypothetical substances of an antienzymotic nature.

As to one other so-called antibody, that of agglutination, Widal came to the undoubtedly correct conclusion several years since that this phenomenon was not a reaction of immunity, but a reaction of infection. Considering the agglutinable substance of the bacterial culture not as an essential part of the bacterial cell, which is undoubtedly a correct conclusion, but as a probable excretory product of the bacterium, just as the toxin is a secretory product, this agglutino-gen might, by a certain possibly excusable laxity of the designatory sense, be included among the antigens; while the agglutinin might be regarded as an antibody, particularly as, like the antibodies, it seems to be formed in the blood, and in that it also has no bacteriolytic action.

Need it be stated here that any bacteriolytic action attributed to the antibodies, antitoxin, like so many other Teutonic emanations, scientific and otherwise, has been almost entirely taken for granted! Indeed, the weight of evidence seems to be much in favor of the antibodies being specifically and indirectly responsible for the production of a nonspecific immunity in infections caused by toxin producing bacteria through tolerance—not by enhancing this condition, but by reducing the amount of poison being liberated in the infected organism, through neutralizing the toxin whose cleavage action on the body cells is producing it, to a point below the tolerance of the subject, thus rendering tolerance efficient.

As far back as 1902 it was demonstrated in Vaughan's laboratory<sup>s</sup> that immune serum, serum from the sensitized animal, had no bacteriolytic action not also possessed by normal serum, despite the fact that the immune serum contained abundant "antibodies"—possessed high powers of agglutinating the homologous bacteria. Yet, without exception, the critics of the theory of protein sensitization still cite the fact that no specific proteolytic action can be demonstrated in blood serum as more or less conclusive evidence that there are no such bodies as specific proteolytic enzymes. The extreme probability that it was this early recognized lack of evidence of the presence of such ferments in the serum, coupled with overwhelming evidence of their existence elsewhere parenterally, which was more or less responsible for Vaughan's theory as it stands, and has stood, since its promulgation, seems to be entirely overlooked.

The theory of protein sensitization, be it understood, was not evolved to explain the mechanism of anaphylactic shock, but to explain immunity; and, although it was tentatively considered by its author as offering a possible explanation of this dreaded experimental phenomenon, it has not been materially affected by any subsequent work—the sensitized animal is particularly liable to



shock, whether it is produced specifically or nonspecifically, and the protein poison must still be rated as at least one of the *substances dechainantes* of our French confrères.

Let us consider more closely, and as fair samples, the two criticisms of Vaughan's theory of immunity which have already been cited, as to what weight, if any, they should have in refuting his advancements.

Jobling and Peterson<sup>9</sup> assume that bacteria are lipoidal in nature, and believe that bacteriolysis is due to the lipolytic effect of the blood serum, the bacterial protein then going into solution. And, as they explain the toxicity of both the injection and reinjection of protein by its adsorption of serum anti-ferment, with a consequent toxic autodigestion of serum protein, and state that an increased resistance to digestion is conferred on the protein of the injection thereby, sensitization, according to this view, can be considered only as an increased ability of the blood to digest itself in the presence of the homologous protein of the injection, which latter is itself protected from destruction by the same process. It may be added that a protein in solution is still a protein, and must be taken care of whether for the purpose of nourishment or for that of elimination.

Much work in Vaughan's laboratory,<sup>10</sup> done years before this, had demonstrated that bacteria are essentially protein in nature, and that fats and waxes make no part of this protein; while work in the same laboratory had early established that the cell residues either sensitize without increasing the capability of the serum anti-ferment to be adsorbed, or were nontoxic with this adsorption.

Bronfenbrenner,<sup>11</sup> on the other hand, assumes that the specific ferment of Vaughan circulates in the blood stream. He regards sensitization as a specific phenomenon, but considers that its specificity rests not in the ferment, but in the mode of its activation. The treatment injection of protein causes the production of antibodies in the sense of Ehrlich. These specific antibodies unite with the antigen of the reinjection, which causes a radical change in the degree of dispersion of the serum colloids, and the normal, nonspecific serum ferment is activated thereby. Toxic symptoms occur only when this ferment acts upon the proteins of the blood, which it does after large reinjections. When the reinjection of antigen is small, it is digested, but without symptoms of intoxication. These visible signs of intoxication mark the *anaphylactic* animal; while the invisible signs of toxicity mark the *immune* animal—in other words, immunity is the result of what might be called a misdirected anaphylactic shock.

This theory, its author states, explains the phenomenon of the parenteral digestion of proteins, as well as the mechanism of *immunity*, on a basis very similar to that of Vaughan; but nothing could well be farther from the fact. Its most glaring difference lies in its inconsistencies, such as a specific *anaphylaxis* and a nonspecific *antianaphylaxis*, which latter is also considered as a benign condition. Saying nothing regarding the assumption about the specific ferment, this theory does not even stand the test of the behavior of, again, the cell residue of Vaughan, which sensitizes and so, according to this conception, must produce antibodies. Beyond this, however, these "antibodies" fail to function, for the sensitizing "antigen," reinjected in whatever dose, fails to produce toxic symptoms; although it will cause harm to the infected subject without

causing visible signs of injury upon its injection, while its homologous protein may cause great benefit while evincing very marked signs of toxicity.

In effect, as far as the question of immunity is concerned, the methods employed in arriving at the conclusions of the above two citations are "empirical." Two absolutely well defined and powerful therapeutic agents, which have dissimilar primary effects and somewhat similar secondary effects, are regarded as one single agent; and this is done inconsistently for the reason that, in both instances, the possibility of separating toxic proteins into their two essential component parts, the toxic nonsensitizing and the nontoxic sensitizing, is acknowledged. And, to quote Fischer,<sup>12</sup> "*while we may not despise any good therapeutic procedure just because it is empirical, we have every reason to despise the modern therapist who employs the empirical method where a rational one is at hand.*" The above methods are not rational.

As before stated, the ultimate test of any theory of immunity must rest with its clinical application; and in this respect Vaughan's theory is found far from wanting in comparison with others. In the preface of his "Protein Split Products" this authority makes certain brief statements of points dwelt upon in the volume. Some of these points will be given in conclusion as having been verified clinically with several hundreds of cases of different infections, treated nonspecifically and specifically with different protein substances, from among which the case cited and discussed has been chosen, not for the reason that it is unique as far as evidencing the correctness of these points is concerned, but rather because the patient's amiable attitude towards any possible discomfort due to testing out questionable conclusions allowed these tests to be made in greater number than with any other single case.

*Protein sensitization and bacterial immunity, apparently antipodal, are in reality identical.*

*Vaccines are protein sensitizers.*

*When proteins are subjected to the action of disrupting agents, as when introduced parenterally, there is the possibility of the chemical nucleus being set free more or less completely, and to the extent that it is detached it becomes a poison.*

*The protein poison is not specific.*

*The tolerance which may be secured by the protein poison is not specific.*

*The sensitization developed by a protein is specific, but is not due to the poisonous group of the protein.*

*The poison elaborated in all infectious diseases is the same.*

#### BIBLIOGRAPHY

- <sup>1</sup>Miller, Joseph L.: The Nonspecific Character of Vaccine Therapy, Jour. Am. Med. Assn., Ixix, 765.
- <sup>2</sup>Vaughan, Victor C.: Protein Split Products in Relation to Immunity and Disease, vi and vii, Lea & Febiger, 1913.
- <sup>3</sup>Idem, p. 51.
- <sup>4</sup>Gay, Frederick P.: Injection of Sensitized Typhoid Vaccine Sediment, Jour. Lab. and Clin. Med., ii, 785.
- <sup>5</sup>Idem.: Page 800.
- <sup>6</sup>Idem.: Footnote, p. 795.

- <sup>7</sup>Vaughan: Protein Split Products, page 306, citing Friedberger and Schymanowski in *Zeitschrift für Immunitätsforschung*, xi, 485.
- <sup>8</sup>Idem.: Page 50
- <sup>9</sup>Jobling, James W., and Peterson, William F.: A Study of the Ferments and Antiferments of the Body and Their Relation to Certain Diseases, *Johns Hopkins Hosp. Bull.*, xxvi, 356.
- <sup>10</sup>Vaughan: Protein Split Products, iv and v.
- <sup>11</sup>Bronfenbrenner, J.: Specific Parenteral Digestion and Its Relation to the Phenomena of Immunity and Anaphylaxis, *Jour. Lab and Clin. Med.*, i, 371.
- <sup>12</sup>Fischer, Martin H.: Some Physicochemical Principles in Therapy, *Forchheimer's Therapeutics of Internal Diseases*, vol. 1, chap. 1, D. Appleton & Co., 1915.

## PNEUMONIA AND MENINGITIS\*

BY PAUL G. WOOLLEY, M.D.

*Major, M. R. C., U. S. Army; Epidemiologist, Camp Greene, N. C.*

ALL infectious diseases are potential producers of "carriers." We mean by that that a germ which produces a disease may outlive the symptoms of the disease in the individual and may remain living and reproducing in the body of its host from whom it is given off or excreted. The individual in whom such bacteria live and from whom they are given off we call a carrier. To account for this state of affairs, in which a pathogenic organism thrives within the body of a host, we say that the host has become immune to the organism and that the organism has at the same time become immune to the host,—to its cells and fluids. Between the two organisms there has been developed a reciprocity that permits both to live harmoniously. If, however, these symbiotic bacteria leave the body of their host and gain access to the body of another individual, disease is apt to occur in that second individual.

Sometimes, however a carrier is a person who has not had the disease which his bacterial guest is prone to produce. Such a person is oftentime only relatively immune and any shock may make it possible for the guest to rob the house and even to destroy the host. Shocks which are active in lowering resistance may be such relatively slight things as mild fatigue or chilling.

So there are two types of peace between organisms,—between host and guest,—one, that which follows a struggle; the other, that which seems to be the result of a temporary agreement. The former tends to be more permanent; the latter to be less so, or even very transient. In the case of typhoid the peace is apparently permanent, as it is in scarlet fever. In the case of pneumonia, on the other hand, it is apt to be transient and in force only during the good behavior of the individual. In meningitis, the conditions we do not understand even as well as we do those in pneumonia. If there is a truce between meningococci and host, then those cocci act as do the Huns, striking with no apparent good reason.

It is sometimes believed that the germs of carrier origin are less virulent than those excreted by persons sick of a disease and that transmission from person to person heightens their pathogenicity. So one accounts for the fact observed in many epidemics that the early cases are more benign than those appearing later in the epidemic. In the cases of meningitis and pneumonia, at least, there seems to be no good evidence that this is true. Or, if it be true, then there is evidence in the course of events as we have seen it in Camp Greene, that transmission from person to person did not occur, for in neither pneumonia nor meningitis was there any evidence that the virulence of the organisms was enhanced.

Naturally enough we know most about the carriers of those diseases in

\*Published by authority of the Surgeon General United States Army. Read at a meeting of medical officers of the Fourth Division, Camp Greene, N. C.



which the causative organisms are known. Also where our knowledge of the bacterium concerned in a disease is most complete, there also is our knowledge of the carrier problem most complete. For instance, we know a great deal about the carrier problems connected with typhoid fever and diphtheria. We know less about those connected with meningitis and pneumonia, and next to nothing about those pertaining to mumps, scarlet fever, and measles. From this it follows that the methods we pursue with regard to typhoid fever are based upon knowledge and are therefore systematic, while with regard to pneumonia and meningitis the methods are based upon very incomplete knowledge and are therefore unsystematic and empiric. Just because of this lack of definite knowledge of meningitis and pneumonia it seems interesting to see what can be added to our understanding of it from our experience in this camp and to compare our experience with those in other places where soldiers are gathered together.

These two diseases have been prevalent in the camps and contonments of the United States during the past winter and have been the basis of a great deal of comment both lay and professional. Meningitis particularly has had an intense sentimental effect. Sometimes this was really merited, sometimes not. Unfortunately, a great deal of the excitement which has been an outgrowth of the incidence of both diseases has been due to a sort of general false impression fostered by unfounded individual and unscientific deductions. Not in all cases, of course, but in many. There has been too much of what "my boy says;" too much correspondence by "my boy" on themes which he should never have tackled. He didn't mean to cause trouble, but he did, and it can scarcely be prevented. We have a democratic army and every one outside, and inside too for that matter, thinks he has a perfect right to draw his own conclusions and criticize when and how he pleases. Often it happens that he does not know the whole situation and more often he is not fitted to discuss the situation even if he knows it. A great many persons have been unable to realize that under the very unusual weather conditions which prevailed during the past winter the only wonder is that there was so little sickness in the camps; not that there was so much. By all the usual rules and suspicions meningitis and pneumonia should have been far more widespread than they were. Measles complications might have run riot. That this did not happen at Camp Greene was due largely to the Base Hospital, a most unusual institution, which in spite of its youth has been extraordinarily efficient.

Suppose we leave these glittering generalities and get down to cases, considering for the moment pneumonia.

There are two types of pneumonia that give us trouble, lobar and lobular. The latter, especially under camp conditions, is a bronchopneumonia. In Camp Greene during November and December there were but seven cases of bronchopneumonia on register cards, all of them in December. At the same time there were forty-one cases of lobar pneumonia, none of them complicated. In January there was but one register card with the diagnosis bronchopneumonia on it. There were, however, several cases of empyema, suppurative bronchitis with empyema, and suppurative bronchitis with bronchopneumonia, all of which were probably originally cases of bronchopneumonia with complications which masked

the primary condition. In January there were register cards for 156 cases of lobar pneumonia, eight of them complicated. One of the complicated cases is of unusual interest because in it the pneumonia was associated with an acute general peritonitis not of intestinal origin. The combination suggests a primary pneumococcus septicemia with secondary attack upon the lungs and peritoneum.

If we study the records for complicating pneumonias we find an opposite state of affairs, in that here the bronchopneumonias predominate over the lobar. For instance, take measles. In November there were no pulmonary complications. In December there was twenty-one, one a bronchopneumonia. In January, measles was complicated in 55 cases, in 13 of which there was a bronchopneumonia.

These complicating pneumonias are most commonly due to streptococci and are the ones which are most apt to be associated with empyema. Personally I feel that when empyema appears in measles, there is a pneumonic process underneath the pleura—that a bronchopneumonia has preceded the appearance of pus in the pleural cavity. The data would be interpreted to mean that of fifty-five cases of complicated measles a streptococcic bronchopneumonia appeared in twenty. It may also be that some of the cases diagnosed lobar pneumonia were in reality bronchopneumonias. This is quite possible because in not a few cases the lobular consolidation is so diffuse that the physical findings approximate those of lobar pneumonia.

Irons and Marine (Jour. Am. Med. Assn., 1918, lxx, 687) call attention to the fact that the physical findings were misleading until an exploratory needle showed that the dullness, thought to be due to lobar consolidation in some of their cases, was really due to the presence of an exudate in which streptococci were found. Also I know from observation at the Base Hospital and from Captain Placak that some, at least, of the cases diagnosed lobar pneumonia were found at autopsy to be massive, or better, diffuse, lobular pneumonias. These are sometimes called pseudolobar pneumonias.

Now because these lobular pneumonias are complications or sequels of other diseases, and since it is only the lobar type that occurs in epidemics, it is the lobar type about which a large amount of work has centered. When we speak of the pneumonia problem in a community we mean lobar pneumonia.

For many years a group of men, particularly in the Rockefeller Institute, have devoted themselves largely to a study of pneumonia. From their researches a valuable mass of facts has accumulated, most of which in condensed form is available in Monograph 7 of the Institute. Among other things these workers have done, has been to show, by means of immunity reactions that there are four main types of pneumococci which they call Type I, Type II, Type III and Type IV. By bacteriologic studies of large numbers of cases of pneumonia they have shown that,—

Type I	is present in	33%	of the cases
Type II	" " "	33.5%	" " "
Type III	" " "	13%	" " "
and Type IV	" " "	20%	" " "

They also have shown that Types I and II are responsible for the most

malignant forms of pneumonia and also that they are the least common inhabitants of the mouths of healthy persons. For instance,—

Type I	occurred in	0.8%	of healthy mouths
Type II	“ “	18.2%	“ “ “
Type III	“ “	28%	“ “ “
Type IV	“ “	52.9%	“ “ “

In other words, Types I and II which account for over 66 per cent of the cases of pneumonia are present in but 19 per cent of healthy mouths, while Types III and IV which cause about 33 per cent of the cases of pneumonia are found in 81 per cent of healthy mouths. These data were derived from bacteriologic examination of 297 mouths of healthy persons, 116 of which were infected with one or another type of pneumococci.

If one is willing to accept these figures, then the logical thing to do in waging war on pneumonia is to guard against the carriers of Types I and II, by discovering them bacteriologically and isolating them until they are free. Also since these types are very commonly found in the environment of patients suffering with pneumonia of a severe character, these patients should be treated just as they would be were they suffering from any other sort of infectious disease, i. e., by isolation and by antiseptic methods applied to the patients and to the objects in their immediate environment.

Now very obviously such a method of dealing with pneumonia, while it can readily be applied to patients in hospitals and to small groups of persons, can not so readily be applied to large bodies such as we find in our camps and cantonments. Nevertheless something like it has been done in England, where, according to Evans (*New York Med. Jour.*, Feb. 2, 1918, p. 220) they make a bacteriologic examination of all recruits, of all patients, and of all contacts; isolate all those found infected, and sterilize all infected mouths. In following such a plan there are chances of error of course. Cases in which infection is localized in the nasal passages where the swabs can not be introduced will be missed. And then also, if the statistics of the Rockefeller Institute are correct, something like 116 men out of 297 would have to be isolated during disinfection for pneumococci alone. How many more would have to be treated in the same way for meningococci we can not say. Or if we should only attempt to disinfect carriers of only Types I and II, then two-thirds of the 116 cases would be concerned. This limitation to these two groups would however not be safe for Miller at Camp Dodge, (*Jour. Am. Med. Assn.*, February 23, 1918, p. 564) had not a single death from patients infected with Type I, and the majority of his cases harbored Types II and IV. And there you are! If you try to collect the various experiences reported from the different camps, you apparently have to conclude to isolate and disinfect all carriers of pneumococci if you wish to stay safe. Personally I believe, in the exigencies of the general situation that it were better to treat *all* recruits in the same simple way. By that means we could learn less about pneumococci types, but the important thing to do, as Vaughan has said is to prevent pneumonia. We are running an army, and not a research institute, and what research we do is a side issue for the time being. We should send all recruits to a camp where for a certain period they would start

their training, and during that time I should attempt to disinfect as thoroughly as possible the upper respiratory passages without regard to the types of bacteria in them. Such a method is in use in our Casual Camp No. 1, and it seems to be effective. The results of the practice are not clear, however, because the general conditions are good. The reports seem to show that there is less pneumonia in the Casual Camp than in the whole camp. Also measles and mumps which in the Casual Camp appeared in contacts from other camps, seem to be declining more rapidly than in the rest of Camp Greene. All this may be an artefact, but it is suggestive, and points to the tentative conclusion that in attacking the pneumococci and meningococci in the nasal passages all the infections of the upper respiratory tract are diminished.

The mere presence of pneumococci in a nose or throat is of course not the whole story. There are many persons who harbor them for long periods of time without discomfort. Invasion and pneumonia depend upon the relative resistance of host and parasite. Factors which produce such decreases of resistance are fatigue due to whatever cause, chilling, and the like. Also a person may be relatively immune to his own nasal menagerie and not to that of another person. He may harbor one type and acquire a second, and the two together, or the second alone, may produce disease. Such secondary infections (co-operating infections) may be frequent in pneumonia. When a primary pneumococcus infection is complicated by a streptococcus infection, the results are yet more severe. Under such conditions it were best to attempt in prophylaxis to limit the number of types of organisms, and so prevent mixed infections. Every man should live only with his own flora. (That holds in many social directions.)

The pneumonia problem of the camps has not been one concerned only with pneumococci, for if we take the reports of Cecil and Sackett (*Jour. Am. Med. Assn.*, 1918, lxx, 728), and of Irons and Marine, we are led to believe that the streptococci must have played an exceedingly important role, not only in the group of primary pneumonias, but also in the measles-pneumonia, and pneumonia-empyema groups. For instance, in Cecil's series of pneumonias 67.7 per cent were due to pneumococci, 24.7 per cent to streptococci, 2.2 per cent to *B. influenzae* and 5.4 per cent to the *M. catarrhalis*. Of the streptococcus cases, 24 per cent were of the hemolytic type. The mortality in streptococcus cases was over 30 per cent. The general mortality for pneumonia was 15 per cent. Empyema occurred in 12.9 per cent of the pneumococcus cases; in 24.9 per cent of the streptococcus cases.

Hackett, speaking of measles in Camp Upton, says that this disease was complicated by pneumonia in 35 per cent of the cases, and that streptococci were responsible for 17.6 per cent of the complications. At Camp Custer, according to Irons and Marine, many of the pneumonias were of streptococcal origin, therefore frequently lobular in type, and frequently complicated by empyema. They examined sputa for pneumococci and found none which agglutinated with sera of Types I, II, or III. During the period of acute respiratory infections, large numbers of throat cultures were made and 70 per cent of the men examined showed hemolytic streptococci, which showed a wide distribu-



tion of these organisms even in apparently healthy soldiers. Irons and Marine say that their "experience with these streptococcal infections suggests that while

	Nov.	Dec.	Jan.
Bronchitis, acute catarrhal	41	231	132
Bronchitis, acute suppurative	1	6	6
Bronchitis, ac. supp. with pneumothorax			1
"    "    "    "    lobar pneumonia			1
"    "    "    "    empyema and pericarditis			1
"    "    "    "    bronchopneumonia			1
Bronchitis, chronic catarrhal			3
Bronchopneumonia		7	1
Lobar pneumonia	7	34	148
"    "    "    with empyema			4
"    "    "    "    otitis media			2
"    "    "    "    ac. general peritonitis			1
"    "    "    "    ac. meningitis			1
Empyema			3
"    "    with pericarditis			1
Laryngitis, acute	4	92	32
Pharyngitis, acute	8	21	15
Influenza	9	197	171
Tonsillitis	71	139	152
Peritonsillar abscess	1	4	10
Vincent's angina	1	1	3
Rhinitis, acute	4	7	8
Otitis media	1	13	23
Sinusitis, ethmoiditis, mastoiditis	2	5	13
Totals	150	847	660
Measles, uncomplicated	82	293	450
Measles, complicated by—			
Acute bronchitis or rhinitis	2		
Influenza	1	1	1
Bronchitis, ac. suppurative	1	2	6
"    "    "    "    and bronchopneumonia	0	1	3
"    "    "    "    "    empyema	0	1	5
"    "    "    "    "    empyema and influenza	0	2	0
Bronchopneumonia	0	0	3
Empyema and bronchopneumonia	0	0	7
Pyopneumothorax	0	0	1
Lobar pneumonia	0	0	2
Otitis media	0	0	10
Tonsillitis	0	0	12
Sinusitis, mastoiditis, ethmoiditis	0	0	2
Empyema, supp. bronchitis, otitis media	0	0	1
Pleuritis, acute fibrinous	0	0	1
Pulmonary tuberculosis, purulent pericarditis	0	0	1
Totals	85	314	505
Pleuritis, acute	4	11	21
Rheumatism, acute	8	20	20
Totals	12	31	41

measles is an important predisposing factor, other infections, as bronchitis, tonsillitis, diphtheria, and other conditions such as exposure, and excessive fatigue must be included—in short anything that may reduce resistance to infection by an organism quite generally distributed during the epidemic of colds and bronchitis; in the noses and throats of the apparently healthy as well as the sick."

These data show distinctly that the prophylactic attack on pneumonia must

not be limited to the pneumococci. At Camp Greene the records indicate in a very suspicious manner that the pneumococci were not all-important or even exceedingly important. By going over the register cards at the Base Hospital I have been able to collect the following data which are of some considerable interest, with respect to the probable types of infection in the respiratory disease. The months of November, December, and January only are given, because in the first place I was interested in collecting data from the early part of the period during which the respiratory diseases rose, and comparing it with the later periods, and secondly because the later files are not complete. Even as it is, there are numerous instances in which the cards for December and January are not completed. The figures are therefore not exact, though they are nearly enough so to indicate the trend of events.

These figures boiled down show that the acute infections of the respiratory tract were fewer in number in January than in December, except in the cases of pneumonia, and tonsillitis. Measles increased by about 62 per cent in January (over December), and was complicated in 10 per cent as against about 6 per cent in December. In November there were no cases of measles complicated by empyema. In December there were six. In January there were fourteen. In November three cases of measles developed complications, none of them severe. In December, twenty-one cases were severely complicated. In January, there were fifty-five complicated cases. In January, the number of pneumonia cases showed a marked increase both in uncomplicated and complicated cases. Also in January the number of primary empyema cases and of acute pleuritis rose.

These data taken together seem to indicate that in November and December, the respiratory infections were preeminently confined to the upper tract, and that later, in January, they had extended to the lower passages. There can be no doubt in the mind of the observer that the bronchitides became chronic, though the hospital records do not show it. From this the suggestion arises that the lower respiratory tracts of many of the men had lost their normal powers of resistance, and that a new acute infection,—such, for instance, that of measles or lobar pneumonia,—produced not only its proper effects, but also gave opportunity for an acute attack by the agent causing the chronic one.

It seems, therefore, from a study covering three months of respiratory disease, that the time at which to begin the prophylactic attack upon the complications of measles is prior to the time when respiratory infections have become chronic.

#### MENINGITIS

Pneumonia is the real menace of camps. Meningitis however is apt to inspire more fear, and when it becomes actually epidemic there is justification for this. When, however, it appears only sporadically, as it has in Camp Greene during the past winter, it is relatively a minor affair, especially when one compares it with pneumonia and measles, the latter with its complications. Mink (*Jour. Am. Med. Assn.*, Feb. 23, 1918, p. 563) says, "Were it not for the fact that meningitis has always been held to be a serious disease, it would probably not inspire so great consideration." So long as such a belief does not interfere with our efforts to maintain the disease as a sporadic one it may be justifi-

fied, but if it tends in the direction of neglecting measures for prevention, then certainly it is pernicious. It must be kept continually before our eyes that as a rule, the mortality from meningitis is much higher than from pneumonia, especially in untreated cases, and that even in treated cases it is somewhat higher. In a series of 712 cases noted by Flexner, the mortality was 31.4 per cent, just about what it has been in our series in this camp. In untreated cases the death rate may be as high as 85 per cent. In Dopter's series of treated cases the mortality was something over 16 per cent.\*

From the standpoint of the carrier, meningitis is simpler than pneumonia, one reason being that there are fewer persons who harbor the organism. Another reason is that chronic carriers are less common than in either pneumonia or typhoid. The meningococcus seems to show a more marked tendency to disappear spontaneously. Also, different from typhoid and dysentery, the meningococci are not carried, so far as we know, by the hands, or clothing, or by flies or other insects. They are present only in the secretions of the upper respiratory passages, and not in the urine or feces. There is no good evidence that they can live outside the body for any considerable period of time.

One wonders just how many carriers there really are. In certain small groups of persons there may be as many as 70 per cent. On the other hand, in large bodies of men, there may be none. The figure obtained from 4446 examinations in Camp Greene was approximately 0.4 per cent. The technic used by the officers who made these examinations was technically perfect and above suspicion. Medlar (Jour. Am. Med. Assn., Feb. 16, 1918, p. 458) has shown that the number of carriers vary in different months. In November and December, 1917, for instance, he found from 3 to 6 per cent. In January, 1918, the number increased to from 12 to 20 per cent, in 3,000 cultures. What makes the difference? Is it methods of laboratory diagnosis? One is inclined to believe this in some cases. It is, however, more reasonable to believe that it is due to the closer physical association which comes with bad climatic conditions, and the coincidental bad housing conditions. With men huddled together in their tents, or in badly ventilated barracks, every chance is given for transfer of bacteria from one to another.

And yet Mink's statements are very disturbing to such a simple conception. For instance, he says that no cases of meningitis appear among the groups of "incoming recruits," notwithstanding the fact that these men were housed together, separated from the rest of the camp, for from twenty-one to thirty days, and despite the fact that there were large numbers of carriers among them,—sometimes as many as 25 per cent (by culture). Accordingly Mink believes that it is not the barracks life *per se*, nor the presence of carriers *per se* that gives the essential conditions for the appearance of the disease. He calls attention to the fact that after the period of detention the recruits were sent to duty and thereafter they enjoyed all the usual liberties, such as visiting the Y. M. C. A.'s and other places of entertainment. It was after this that the disease appeared, but even then the incidences of the disease and of the carriers did not correspond. In some barracks in which there were 25-30 per cent of

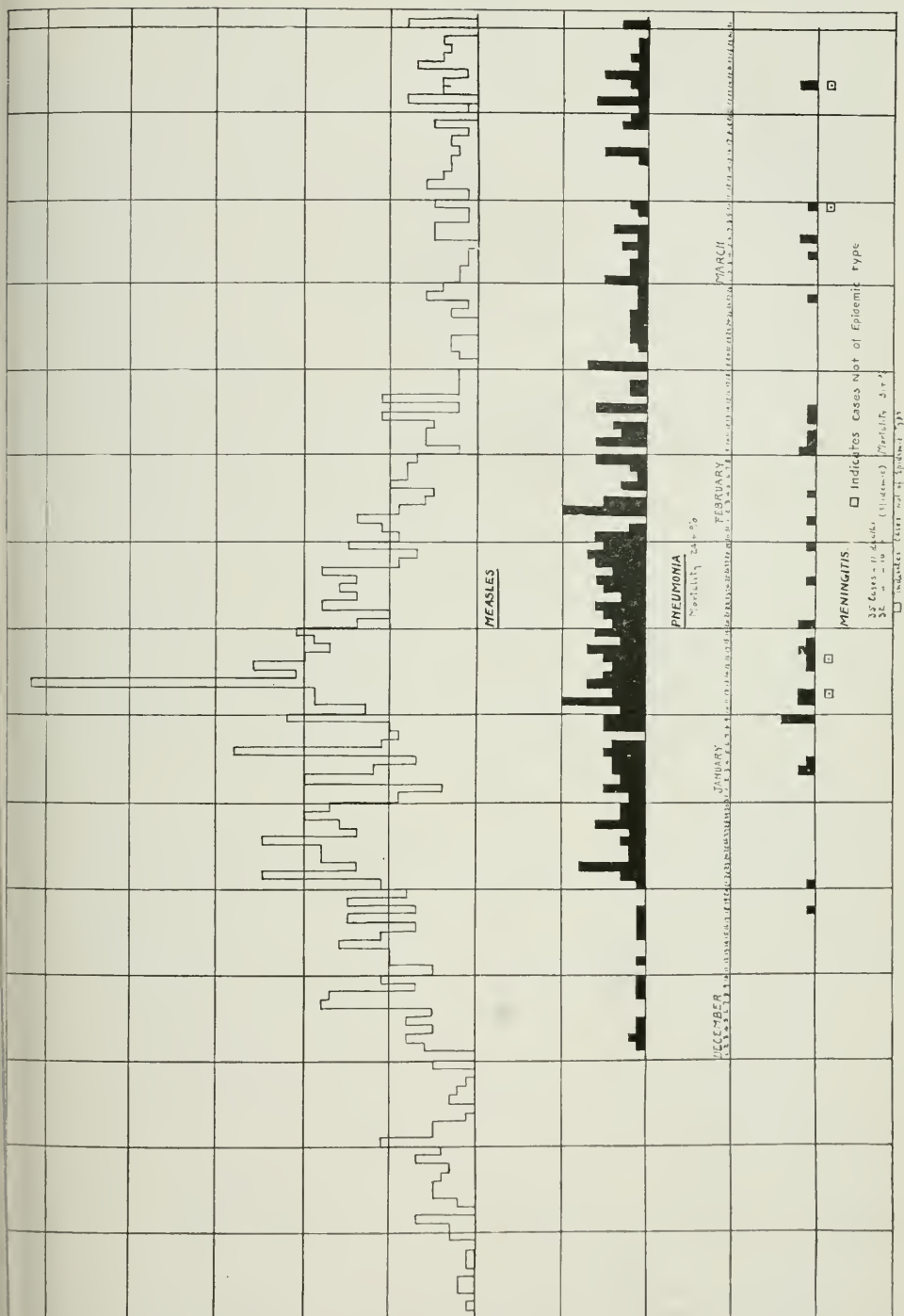
\*Rosenow has recently reported (Jour. Am. Med. Assn., 1918, lxx, 759) a series of pneumonias treated with his vaccine. Among these cases the death rate was but 7 per cent.

carriers there was no meningitis; in others, with 8-9 per cent, the disease showed itself. Yet, in the whole series of cases in the command, the incidence of disease was higher in the carrier group than in those who were not carriers,—a state of affairs completely at variance with the rule. Small wonder that Mink is not impressed by the attempts to control meningitis by the methods based upon carriers! As a matter of fact the mere isolation of carriers seems to have had little to do with the control of meningitis in Camp Greene. So long as the effort was made only to discover and isolate carriers, without at the same time doing anything else, the disease kept reappearing. But in those organizations in which attention was paid as much to cleansing the nasopharynx, as to isolation of contacts and carriers, the disease disappeared. The facts may be the results of coincidence, but they are exceedingly suggestive. So suggestive is it to me that I am almost willing to say that the conditions could have been met and controlled just as successfully if the immediate contacts had been isolated in their own squad tents until they were cultured, and then if they were negative, allowed to drill and take part in all the routine duties of their organizations. At the same time the use of disinfecting sprays should have been used on all members of the company in which the meningitis appeared. I feel that the carrier is a source of considerable danger only when the men of an organization are low in resistance, brought about by fatigue, the result of overexertion or exposure. The men must suffer frequently from fatigue. Therefore the problem seems to center itself upon disinfection of the nasopharynx—the dwelling place of the meningococcus.

It is a fact that up to the present time the local causes of epidemics of meningitis have been sought in vain. Water, milk, food, all have been excluded from consideration. Moreover, as Frost says, the disease "does not present epidemiologic characteristics which we are accustomed to associate with a contagious disease. Even in carefully studied epidemics it is the exception rather than the rule to find any direct or even traceable indirect contact between successive cases." In Camp Greene there has been but a single case in which suspicion of contact could be discovered. Also, "it is unusual to find more than one case of the disease in a family, and when multiple cases do occur in the same house, they often occur so close together, or separated by such a long interval as to make it seem unlikely that one was infected from the other." In the Camp Greene series of cases the same statement can be applied to perfection. In a single instance more than one member of a company was attacked by meningitis, and in no case did more than one case appear in the same tent. These facts render it virtually certain that "direct contact with the sick is neither necessary, nor even a very common factor, in contracting the infection."

The experience of Mink in regard to the progress of his epidemic of meningitis is strikingly similar to ours in Camp Greene. Mink says that the first thing in the sequence was an outbreak of coughs and colds so extensive that almost whole regiments were crippled with bronchitis, coryza and influenza. Four days later, the first case of meningitis appeared. So long as these respiratory difficulties persisted, meningitis did also, and finally when bronchitis and influenza subsided, so also did meningitis. I have not been able to get concise data on conditions as they existed in November during which two cases of





Daily incidence curves of disease in Camp Greene. 1 square = 1 case.

meningitis appeared, but the conditions since that time appear to simulate those of Mink. The chart shows graphically the number of cases of meningitis, pneumonia, and measles, by days, during December, 1917, and January, February and March, 1918, and demonstrates very clearly, it seems to me, the heaping up of these diseases during January, a month in which living conditions were at their worst. It was these months also in which the complications of measles and pneumonia were so frequent. (See table.) In this connection Frost says, (Public Health Reports, No. 26, 1913) "the seasonal prevalence (of meningitis) is strikingly similar to that of pneumonia and influenza, and not unlike that of a number of other diseases, (scarlet fever, measles, diphtheria and smallpox) in which the primary seat of infection is believed to be the respiratory tract." If this be true that the incidence of meningitis and that of the commoner respiratory diseases are parallel; if, in fact, the appearance of meningitis follows the rise in acute respiratory diseases, then one may quite reasonably draw the inference that there is some definite connection between the two. Medlar believes that meningitis is primarily a nasopharyngitis. One interpretation of that is that a nasopharyngitis of nonspecific character offers the opportunity for penetration of the mucous membranes by meningococci. It is a well-known fact of general pathologic interest that an inflamed mucous membrane offers special inducements to bacteria to pass through it. One reason for this is that there are increased numbers of phagocytes present upon inflamed surfaces, and these engulf microorganisms and carry them inward. Another reason is that the mucous membranes are colloidal structures which when swollen are more dilute and have less than a normal viscosity. Under such circumstances any particle may pass through them more readily than while they retain their normal water content.

It deserves mention that it seems probable that epidemic meningitis is not primarily an inflammation of the meninges, but is a septicemia in which the bacteria tend to become localized in the membranes of the brain and cord. This conception has been emphasized by Herrick in his remarks on meningitis at Camp Jackson (Jour. Am. Med. Assn., Jan. 26, 1918, p. 229). This is what might be expected from the clinical picture, especially in the fulminating cases, and from a consideration of the course the cocci would have to take to reach the meninges. That the bacteria must frequently be present in large numbers, and at a very early stage, in the blood is tested by the very early appearance of the petechial eruption from which the term *spotted fever* comes.

In conclusion, all that seems possible to say with regard to pneumonia and meningitis prophylaxis, is that both are due to invasion of bacteria which are inhabitants of the upper respiratory tract, that the conditions under which they become more or less widespread or epidemic are not understood, and that the only method for preventing their spread is to apply antiseptic methods to the mouth and nasopharynx. It seems obvious that the application of such methods should be put into practice in advance of the season of the year during which the incidence of respiratory diseases rises.

## BIBLIOGRAPHY

- Frost: Epidemic Cerebrospinal Meningitis, Public Health Repts., U. S. P. H. S., No. 69, 1913.
- Editorial: Meningococcus Carriers, Jour. Am. Med. Assn., 1918, lxx, 781.
- Blevins: Report of Seven Cases of Epidemic Meningitis, Military Surgeon., 1917, xli, 646.
- Ezdorf: Epidemic Cerebrospinal Meningitis, Public Health Repts., No. 124, 1913.
- Baesslack, Bunce, et al: Cultivation of Meningococcus, etc., Jour. Am. Med. Assn., 1918, lxx, 684.
- Park: Communicable Diseases Among the Soldiers in England and France, New York Med. Jour., 1918, Feb. 9, p. 268.
- Medlar: Epidemic Cerebrospinal Meningitis at Camp McClellan, Jour. Am. Med. Assn., 1918, lxx, 458.
- Thomson & Wulff: Meningococcus Infection and Meningitis, Hospitalstidende, 1917, lx, No. 49, (Abstr.).
- Mink: Epidemicity of Meningitis, Jour. Am. Med. Assn., 1918, lxx, 563.
- Editorial: Endemic and Epidemic Meningococci, New York Med. Jour., 1918, Feb. 23, p. 369.
- Irons and Marine: Streptococcal Infections Following Measles and Other Diseases, Jour. Am. Med. Assn., 1918, lxx, 687.
- Cecil: Pneumonia in Camp Upton, Jour. Am. Med. Assn., 1918, lxx, 729.
- Hackett: Measles in Camp Upton, Jour. Am. Med. Assn., 1918, lxx, 728.
- Evans: Problems of Communicable Diseases in Training Camps and Cantonments, New York Med. Jour., 1918, Feb. 2, p. 220.
- Knopf: Pneumonia Among Soldiers in Camps, Cantonments and at the Front, Ibid., Jan. 26, p. 165.
- Pneumonia: Its Prevention and Treatment, Jour. Am. Med. Assn., 1918, lxx, 382.
- Kolmer and Steinfeld: Disinfection of Pneumococcus Carriers, Jour. Am. Med. Assn., Jan. 5, 1918.
- Avery, Chickering, Cole, and Dochez. Acute Lobar Pneumonia, Monograph No. 7, Rockefeller Institute, Oct. 16, 1917.
- Alexander: Hemolytic Streptococcus Causing Severe Infections at Camp Zachary Taylor, Jour. Am. Med. Assn., 1918, lxx, 775.
- Rosenow: Partially Autolyzed Pneumococci in the Treatment of Lobar Pneumonia, Jour. Am. Med. Assn., 1918, lxx, 759.
- Vaughan: Measles and Pneumonia in Our Camps, Jour. Lab. and Clin. Med., 1918, iii, 248.
- Duncan: Measles and Pneumonia at Camp Wheeler, Mil. Surg., Feb., 1918, xlii.

## ON THE RELATION OF PEPTONE TO BIOLOGICAL REACTIONS\*

BY WALTER A. JAMIESON, INDIANAPOLIS, IND.

WITH the outbreak of the war and the consequent curtailment of the supply of Witte's peptone, the question of the production of potent diphtheria toxin became acute. American peptones already on the market, while satisfactory for the production of tetanus toxin and allowing a profuse growth of *B. diphtheriæ*, did not give a satisfactory toxin production. Other American peptones were soon available which gave a diphtheria toxin of low potency.

Of the most of the American peptones one lot might give a toxin of 400 to 600 M.L.D. per mil, while the next lot might give no toxin at all. Within the last year and a half one of these peptones, however, has given fairly constant results.

A peptone was made up as nearly as possible of the same composition as Witte's peptone, with some variation, however, which there was reason to believe would make it a better peptone than Witte's. Toxin was produced with this peptone, using as comparison Witte's peptone and American brands. In all cases the new peptone gave the most potent toxin, and Witte the next highest. Inasmuch as consistent results were secured with but one of the American peptones, this was used for comparison with Witte's peptone and the special peptone studied.

The toxin produced with the special peptone has tested as high as 1000 M.L.D. per mil; it consistently tests 500 to 600 M.L.D. per mil. The Witte peptone has tested about 500 M.L.D. per mil, while the American brand has made toxin fluctuating between 100 and 300 M.L.D. per mil.

Experiments were made to determine whether the peptone employed in the toxin production had a detrimental effect when injected into animals; or whether the peptone was such that the growth of the diphtheria organism would produce by-products which were injurious when injected into animals.

Two series of guinea pigs were injected, one with broth before inoculating it with *B. diphtheriæ* for the production of toxin, another with toxin neutralized with an excess of antitoxin, using in each case lots prepared with Witte's peptone, the American brand, and the special peptone. Two guinea pigs were inoculated in each series with the material from the three peptones.

Table I shows the results of the injections of the peptone broth and Table II shows the results of the toxin-antitoxin experiment.

In Table I it will be noticed that the pigs injected with Witte's peptone showed no early ill effects but that during the period of the fifth to the seventh injections both suffered a drop in weight. With the American peptone one of the pigs lost weight steadily, finally became paralyzed and died; the mate first steadily lost in weight then gradually gained a little, and remained constant. The

\*From the Biological Laboratories of Eli Lilly and Company, Indianapolis, Ind.



TABLE I\*

DATE	INJECTION	WITTE'S PEPTONE		AMERICAN PEPTONE		EXPERIMENTAL PEPTONE	
		WT. PIG 268	WT. PIG 205	WT. PIG 206	WT. PIG 202	WT. PIG 263	WT. PIG 264
11-17	0.01 c.c.	250	280	250	215	295	280
11-19	0.02 "	235	265	235	195	285	260
20		245	275	240	210	315	280
21	0.04 "	245	270	225	200	300	275
22		245	275	225	195	295	280
23	0.08 "	240	260	220	190	285	270
24		230	260	215	200	280	260
26	0.16 "	210	250	205	180	275	275
27		225	255	205	180	260	265
28	0.32 "	215	245	195	165	260	260
30	0.64 "	210	235	175	170	250	260
12-1		200	235	185	170	255	260
3	1.28 "	240	255	185	180	300	275
4		235	260	175 paral.	180	290	275
6	1.5 "	260	280	Dead	200	310	305
7		255	285		205	310	300
8	1.5 "	250	260		200	290	285
10	1.5 "	245	265		180	290	265
11		265	290		215	290	275
12	1.5 "	270	285		220	310	310
13		265	280		220	335	300
14	1.5 "	270	285		200	330	305
15		275	290		210	320	300
16	1.5 "	260	285		200	325	310
17		265	280		205	320	305
18	1.5 "	260	275		215	315	315
19		265	275		210	315	320
20	1.5 "	260	270		210	310	315
21		255	270		210	305	315

\*Injections consisted of samples from broth which had been sterilized and was ready to be inoculated with *B. diphtheriae* for the production of toxin. The first five injections were made up to a volume of 0.5 c.c. by the addition of NaCl 0.85 per cent.

pigs on the special peptone showed a little loss of weight from the fifth to seventh injections and then rapidly increased in weight.

In Table II it will be seen that one of the pigs on Witte peptone steadily lost weight and finally died, whereas the mate kept fairly constant weight throughout the experiment. On the American peptone one pig constantly lost weight, finally became paralyzed and died. The mate, except for one drop in weight, remained about constant. Both the animals on the special peptone kept fairly constant weight.

In both experiments the danger period seemed to be from the fifth to eighth or ninth injection. The pigs on the special peptone did not lose as much weight and were more lively than the other pigs. Of those on Witte's peptone one died in the second experiment and one lost a great deal of weight in the first experiment. Of those on the American peptone one died in the first experiment while the other lost weight, and this was the case also in the second experiment; both the pigs which died developed a complete paralysis of the hind quarters which extended to the whole body.

While these tests are too few in number to be conclusive, they seem to

TABLE II\*

DATE	INJECTION	WITTE'S PEPTONE		AMERICAN PEPTONE		EXPERIMENTAL PEPTONE	
		WT. PIG 265	WT. PIG 262	WT. PIG 210	WT. PIG 203	WT. PIG 267	WT. PIG 270
11-17	0.01 c.c.	280	275	280	260	270	270
19		265	280	265	245	265	265
20	0.02	270	290	285	260	275	280
21		270	290	290	250	270	270
22	0.04	260	290	275	255	270	280
23		255	280	280	250	265	265
24	0.08	250	270	280	245	260	255
26	0.16	245	270	270	240	250	250
27		250	270	270	240	250	255
28	0.32	240	265	260	225	250	250
30	0.64	230	265	250	220	250	245
12-1		225	250	245	215	240	245
3	1.28	200	285	235	250	265	270
4		Dead	270	230 paral.	245	255	275
6	1.50		305	not 220 inj.	270	260	310
7			305	Dead	270	255	290
8	1.5		295		260	240	270
10	1.5		285		245	225	255
11			320		280	225	280
12	1.5		315		275	230	300
13			320		280	230	300
14	1.5		320		275	225	305
15			315		280	230	305
16	1.5		300		280	235	300
17			290		285	230	310
18	1.5		285		270	230	290
19			290		275	235	285
20	1.5		285		285	230	280
21			290		280	230	275

\*Injections consisted of toxin neutralized by the addition of an excess of antitoxin. The material for all the injections was made up at the beginning of the experiment. The first five injections were made up to 0.5 c.c. by the addition of NaCl 0.85 per cent.

indicate that the toxicity of the special peptone is decidedly less than the American peptone and as low if not slightly lower than the Witte peptone.

The relationship of the  $L_+$  dose to the M.L.D. of the toxins made from the three peptones was next determined. The ideal ratio of the M.L.D. to the  $L_+$  dose is of course 1:100. By testing out the M.L.D. and the  $L_+$  dose of toxins from the three peptones the following ratio of the M.L.D. to the  $L_+$  dose was found:

Witte's peptone, 1:85  
 Special peptone, 1:110  
 American peptone, 1:150

One toxin from the American peptone gave a ratio as high as 1:180, the M.L.D. dose being 0.0025, the  $L_+$  dose 0.4 c.c.

It should be noted that the Witte's peptone toxins were six months old or more before being tested while the special and American peptone toxins were fresh when tested. From these results it seemed that the special peptone produced a toxin which was more nearly ideal than the American brand.

## OTHER BIOLOGICAL REACTIONS

The results given led to the belief that other biological tests were being confused by the use of different peptones. While the step into this field has been scarcely taken, it seems advisable at this time to report one or two instances that may lead to further investigation by others with the hope that if varying results are being obtained due to the use of different brands of peptone, some standardization may take place as regards peptones, to eliminate if possible at least one of the many errors to which most biological reactions are subject.

Several lots of typhoid vaccine were made up on mediums made from an American peptone (other than the American peptone employed in the production of diphtheria toxin). This vaccine was injected into rabbits and the rabbits then bled and their blood tested for agglutinin, using an antigen prepared from cultures grown on the same peptone. It was found that apparently no agglutinins were present.

However, using an antigen prepared from Witte peptone, agglutinins were found in the usual quantity while with antigens prepared on four other American peptones the results were negative except in the case of one American brand which showed agglutinins to be present in about half to three-fourths the amount shown by Witte's peptone. The results were obscured in the case of cultures on two of the American brands by the fact that they seemed to be spontaneously agglutinable. Using the special peptone, the same which was employed in the production of diphtheria toxin, the results showed that agglutinins were present in approximately the same quantity as those obtained with cultures on Witte's peptone.

The same was true with agglutination tests with meningococcus serum. The animals were injected with cultures grown on mediums made with American peptone. The serum showed a low agglutination titer when tested with cultures grown on American peptone media. With the same cultures carried on Witte's peptone and special peptone the serums showed a much higher agglutination titer.

## CONCLUSIONS

Care should be exercised in the selection of peptone for the production of diphtheria toxin inasmuch as the peptone used may influence the physical condition of the animal injected with the toxin.

The peptone employed in the production of diphtheria toxin influences the potency of the toxin and what is of greater importance the ratio of the constituents of the toxin as shown by the ratio of the M.L.D. to the  $1+$  dose.

The peptone used in the production of antigens employed in immunologic tests will influence the results of the tests.

# LABORATORY METHODS

---

## RECOMMENDATIONS OF THE COMMITTEE ON A STANDARD ROUTINE METHOD FOR THE ISOLATION AND IDENTIFICA- TION OF HEMOLYTIC STREPTOCOCCI FROM THROATS, SPUTA, AND PATHOLOGIC EXUDATES\*

IN making these recommendations the committee is confronted by the necessity of sacrificing something in the direction of thoroughness and detail in order to recommend a method that will be simple and practical for use on a very large scale under the conditions obtaining in the army camps and cantonments.

The method recommended involves the isolation of the hemolytic streptococcus into bouillon from a blood agar plate and the use of the bouillon culture for various tests.

### COLLECTION OF PATHOLOGIC MATERIAL

Throat swabs (tonsils and pharynx) are to be taken on ordinary sterile cotton swabs encased in sterile test tubes. These are to be sent promptly to the laboratory. Pus, if scant in amount, may also be collected on swabs. Considerable amounts of pus, pleural exudate, or sputum should be collected in sterile bottles or tubes (without antiseptic), taken promptly to the laboratory and there kept cold until cultured.

### BLOOD AGAR PLATES†

*Inoculation.*—Swabs should be moistened with a drop or more of sterile salt solution unless they are obviously quite moist. Sputum should be washed as for the isolation of the pneumococcus and a kernel selected for culturing. A Gram stain of the sputum, swab, or exudate will often help to determine the amount of material that should be used for inoculation of the plate.

It is preferable to study both surface and deep colonies in the blood agar plate, hence material of known importance, such as pleural exudate, pus, and material from autopsies, should be suitably diluted, inoculated into fluid blood agar (at 45-50° C.) and poured into the petri dish. A small bit of the material may also be streaked out on the same or a different plate if desired.

For routine examination of swabs in large numbers surface inoculation only of blood agar plates is sufficient. It is recommended that only one swab be inoculated onto each plate. A convenient method of inoculation is to touch a spot near the edge of the plate with the moist swab and then with a platinum loop to smear the material from this spot back and forth across the surface of the medium with a view to securing greater dilution at points farthest from

\*Report of a committee appointed by the Surgeon-General.

†If streptococci are being sought for in material in which they may be quite rare, a preliminary growth in serum bouillon, dextrose blood bouillon, or cooked meat medium will serve to encourage the growth of streptococci above that of other organisms. If, on the other hand, it is desired to know the relative numbers of streptococci and other organisms present in the original material, it should be plated directly without preliminary "enrichment."



the spot touched by the swab. A cut into the agar by means of the edge of the loop for a short distance soon after it has left the inoculated spot will give some opportunity for growth in the depths of the agar.

*Incubation.*—Blood agar plates should be incubated at 37° C. in an inverted position for 18-24 hours before final observations are made. The atmosphere of the incubator should be kept humid by the exposure of a large surface of water in shallow pans on the floor of the incubator.

*Study of Plates.*—Isolated colonies with well defined colorless zones of hemolysis should be sought. There should be no pigmented (greenish or brownish) corpuscles visible under the low power of the microscope remaining next to or under the colony. The approximate number of such colonies (expressed as a percentage) with respect to all other colonies should be noted.

#### BOUILLON CULTURE

*Inoculation.*—A single typical isolated hemolytic colony is to be transferred to a tube of bouillon.

*Incubation.*—After incubation overnight or until there is a good amount of visible growth the bouillon culture is to be used as follows:

*Study.*—

1. A Gram stain is to be made and examined to make sure that the organism is a streptococcus and for a study of its morphology. The points to be noted are the length of the chains, the size, shape, and arrangement of the cocci in the chains, and any striking peculiarities.

2. A stock culture is to be made if it is desired to keep the strain in cultivation or if there is a possibility of the strain being used for further study later than the next day. If it is desired to save time in procuring the stock culture it may be inoculated from the blood agar plate at the same time the bouillon is inoculated provided the same colony is used, or it may be inoculated with a loop of the bouillon after the latter has been inoculated. The latter method serves to make a dilution of the material from the colony so that isolated colonies are obtained on the slant of the stock culture.

3. 0.5 c.c. of the bouillon culture is to be mixed with 0.5 c.c. of a 5 per cent suspension of washed rabbit blood corpuscles in physiologic salt solution and incubated in a water-bath at 37° C. for 2 hours. Streptococci which laked the blood completely in this time are to be regarded with suspicion. Markedly hemolytic pathogenic streptococci of human origin are known to produce laking of blood under these conditions.

4. To about 1 c.c. of bouillon culture add one-fifth volume of sterile ox bile. Observe for 1 hour at room or incubator temperature. Under certain conditions pneumococci on blood agar may cause hemolysis. Solubility in bile, however, serves to distinguish them from streptococci.

The above procedure is to be regarded as essential. The following additional tests are advised for more detailed study of cultures when time and conditions permit:

5. The bouillon culture to be replated (after proper dilution) into fluid blood agar so that the deep colony as well as the surface colony may be studied and as a check upon the purity of the culture.

6. The fermentation reactions towards lactose, mannite, salicin, inulin, raffinose and saccharose should be determined, the first three or four substances mentioned being of most importance.

7. A tube of milk should be inoculated and incubated for 7 days. During this time it should be noted whether coagulation occurs promptly, slowly, or not at all, and if it has not occurred the tube should be immersed in a boiling water bath for 10 minutes to see whether coagulation occurs under these conditions.

8. Inoculation of rabbits intravenously with not more than 1 c.c. of the fresh bouillon culture and inoculation of mice intraabdominally with much smaller amounts.

## NOTES AND DETAILS

### BLOOD AGAR PLATES

*Agar.*—Standard beef infusion agar of the following composition shall be used:

	PER LITER OF MEDIUM
Aqueous extractives of	500 gms. of meat.
Agar-agar	20 gms.
Peptone	10 gms.
Salt	5 gms.

Final titratable acidity (after sterilization) to be between neutral and 0.5 per cent normal acid to phenolphthalein.

No dextrose or other fermentable substance should be added to the medium.

*Kind of Blood.*—Sterile defibrinated horse, human, or rabbit blood may be used. The sharpest results are probably obtained with horse blood but most hemolytic pathogenic streptococci are readily recognized on any of the kind of blood mentioned.

*Mixture of Blood and Agar.*—The agar medium should be melted and cooled to 45 to 50° C. at which temperature 5 to 10 per cent of blood is added and thoroughly mixed into the agar.

*Pouring of Plates.*—A petri dish 9 cm. in diameter should receive 12 to 15 c.c. of blood agar, i. e., sufficient to make a layer 2 to 3 mm. thick.

*Caution.*—Sterile defibrinated blood may be kept in the refrigerator for several days but should not be used if it has begun to lake or if the sedimented corpuscles have become viscid so that it is difficult to shake them up into suspension.

Poured blood agar plates should not be kept on hand "ready for use" for longer than a few hours under ordinary conditions. If the surface of the medium becomes even slightly dry streptococci will not grow readily on it. If kept too long in the refrigerator the moisture collecting on the surface of the medium or undersurface of the lid is likely to cause confluence of colonies or to encourage the overgrowth of "surface spreaders."

### BOUILLON CULTURE

The bouillon used should be standard meat infusion bouillon of similar composition to the agar described above. No dextrose should be added. No serum or ascitic fluid should be added if the bouillon culture is to be tested for solubility.

in bile. The titratable acidity should be not above 0.5 per cent normal acid to phenolphthalein.

The bouillon culture should be used for the various tests only when it is quite fresh, not after it is two, three, or more days old, nor should tests be conducted by inoculation from one test medium to another. For later tests and experiments fresh bouillon cultures should be made directly from the stock culture.

#### STOCK CULTURES

Streptococci remain viable for many weeks or months on (1) blood agar slants, (2) standard agar slants to the surface of which a few drops of blood have been added, and (3) in bouillon to which a small percentage of blood has been added.

Blood agar slants are conveniently made by pouring fluid blood agar on the surface of previously prepared agar slants. There is obtained a layer of blood agar of uniform thickness on which the hemolytic action of the streptococcus colonies is readily observed for confirmation of the purity and character of the strain.

Dextrose should not be added to any of the stock culture media. Before being used any of the above media should be incubated for 48 hours after the blood has been added, to test their sterility. When slants are used the condensation fluid as well as the surface of the slant should be inoculated with the streptococcus. After inoculation any of these media should be incubated barely overnight and then kept cold.

#### SOLUBILITY IN BILE

Fresh undiluted ox bile may be autoclaved, filtered through paper, and again autoclaved. It is then ready for use as directed above.

#### SHIPMENT OF CULTURES

Cultures on the slant media described above may be shipped but for this purpose most of the condensation fluid should be pipetted off to prevent wetting of cotton plugs during shipment. It is also well to insert a cork stopper into the tube after the cotton plug has been burned off and pushed in.

The committee does not feel called upon to recommend in detail methods for the additional tests numbered 5, 6, 7 and 8 above, but is authorized to state that cultures of special interest may be sent to Rockefeller Institute for Medical Research, New York City, where they will be studied in detail. Each of these cultures should be accompanied by information regarding source, method, and date of isolation, the results of tests already conducted, and the medium on which it is grown.

Signed:

W. L. HOLMAN,  
OSWALD T. AVERY,  
R. A. KINSELLA,  
J. HOWARD BROWN.

June 1, 1918.

## SIMPLIFIED GAS ANALYSIS

BY J. J. R. MACLEOD, M.B., CLEVELAND, OHIO

THE extraordinary development of medical education in recent years, especially in this country, is attributed in large part to the introduction of practical laboratory courses in which simplified and fundamental experiments can be performed by the student himself. When the student is given the opportunity to "discover" some facts for himself, not only is his interest in other facts which he must accept on the authority of more highly trained investigators greatly enhanced, but he is in a better position to correlate them with what he already knows. This training is of value to him not alone in the particular science, such as physiology, to which the methods apply, but also in preparing him to estimate at their true value the signs and symptoms with which he will have to occupy his attention in the clinic. It also gives him a much greater enthusiasm for his work.

In many parts of physiology, particularly nerve-muscle physiology and the circulation, there is an adequate number of sufficiently simple experiments to make the practical courses of great value. But in other parts, this is far from being the case, and in none more conspicuously so than in respiration. To expect the student to comprehend the intricate problems connected with the exchange of gases between the blood and air and between the blood and tissues without himself doing some of the fundamental experiments, is as unreasonable as it would be to expect him to learn clinical medicine away from the patient. In order to make such experiments possible, considerable simplification of the apparatus and technic that have been used by the experienced investigator is essential, and it is proposed in this and subsequent articles to show how this has been accomplished in the classes under the writer's care.

There are two great difficulties in using gas-analysis apparatus with large classes of students. One is the sticking or "freezing" or breakage of stopcocks and the other the large amount of mercury required for many of the processes. In the apparatus used in these experiments stopcocks are almost entirely replaced by screw clips, a device being introduced to equalize the gas pressure in the apparatus after the screw clip has been tightened, and very little mercury is employed. Much of the apparatus, especially that used for the analysis of alveolar air and the total respiratory exchange, can also be used with great advantage for routine clinical work without any significant sacrifice of accuracy.

### NO. I. SIMPLIFIED PUMP FOR REMOVING AND ANALYZING THE BLOOD GASES

The first process to be described, namely, the removal of the gases from blood by a vacuum pump, is in many ways the most complicated of them all and it is one which can have no important clinical application. Nevertheless it is an experiment of very great value in the training of the student, since it demonstrates as no other method can that the blood carries a large amount of gas which is completely evolved only when the pressure is greatly reduced. The



apparatus is adapted from that of Leonard Hill,\* the modifications being as follows:

1. The use of a syringe working in an oil-bath in place of the leveling tube with mercury.
2. The use of a substance to prevent foaming of the blood during the evacuation process, thus permitting a much smaller blood bulb to be used.
3. The collection of the gases in a graduated pipette instead of an eudiometer tube.
4. The preliminary evacuation of the blood bulb by means of one or other of the high-grade vacuum pumps which are usually part of the equipment of a physiological laboratory.

#### DETAILS OF METHOD

Place about 15 c.c. defibrinated ox blood in a 500 c.c. flask, and fill the latter with alveolar air by expiring deeply into it through a piece of wide-bore rubber tubing, or better, through glass tubes inserted through the stopper of a

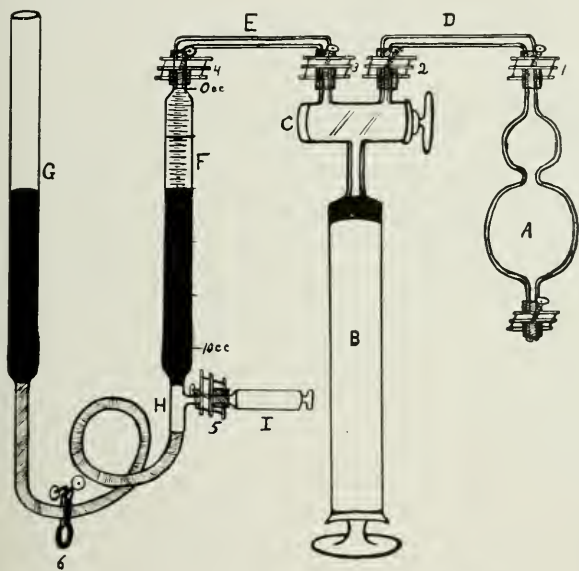


Fig. 1.

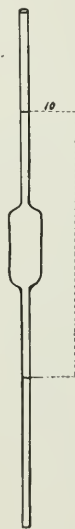


Fig. 2.

bottle filled with glass beads, which condenses and removes the water from the air. Rotate the flask so that the blood forms a thin film on the walls, but do not shake in such a manner as to cause the blood to froth. While rotating, occasionally expire through the tube into the flask so as to maintain the percentage of carbon dioxide constant. Continue this procedure for about three minutes and then close the flask. By this procedure the blood absorbs oxygen to full saturation, and carbon dioxide to the same extent as that of the blood in the pulmonary capillaries.

Meanwhile the bulb of the blood pump (Fig. 1, A) is evacuated by connecting it, by means of the attached piece of rubber tubing, to a vacuum pump (Geryk Cenco-Nelson), which is operated until the manometer records as low a

\*Hill, Leonard: Jour. Physiol., 1894, xvii, 353.

pressure as possible. The screw clip (1) is then tightened, leaving as long a piece of tubing beyond the clip as possible. A few drops of the antifoaming solution (caprylic alcohol or isoamylisovalerate) is then allowed to flow into the bulb. This is accomplished by taking about 0.5 c.c. of the fluid in a narrow-bored glass tube of sufficient external diameter to tightly fit the rubber tubing of the blood bulb (a 1 c.c. pipette with the delivery tapered end partially cut off). Before inserting the pipette into the rubber tubing, the lumen of the latter beyond the clip is filled with the antifoaming solution, so that no air may enter the bulb. The screw clip (1) is then very cautiously opened and about 0.1-0.2 c.c. of the solution allowed to run in, after which the clip is again screwed tight, the pipette removed, and the solution still in it replaced in the stock bottle.

Ten c.c. of blood is now placed in the bulb. To accomplish this remove blood from the flask by means of the special 10 c.c. pipette (see Fig. 2), using only gentle suction and filling to the upper mark. Squeeze all air out of the tub-

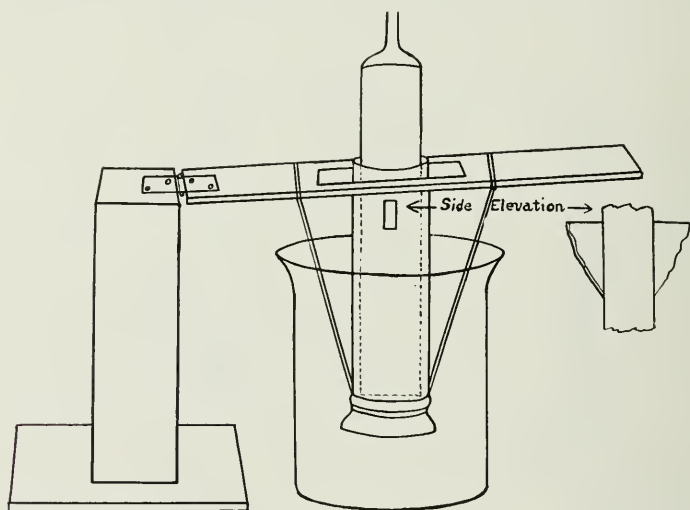


Fig. 3.

ing on the blood bulb, and then insert the end of the pipette in the tubing, being careful to see that no air bubbles are present at the union. Holding the pipette and blood bulb vertically, cautiously unscrew the clip (1) and allow the blood to flow from the pipette into the bulb until the lower mark on the former is reached. The capacity between the two marks is 10 c.c. After tightening the screw clip, remove the pipette and squeeze out the blood left in the tubing.

The blood pump must now be prepared. This consists of a 50 c.c. all-glass (Luer) syringe (*B*), with vaseline between the walls and piston, the nozzle being connected by thick-walled rubber tubing with the single tube of a three-way stopcock (*C*). Of the other tubes of the stopcock, one (*D*) runs to connect, by narrow-bore glass tubing, with the blood bulb and the other (*E*) to the graduated burette (*F*). (A 10 c.c. graduated pipette is satisfactory.) To avoid all risk of air leakage into the syringe, this is manipulated in an oil-bath,\* as shown in Fig. 3. A brass tube of a diameter slightly greater than that of the syringe

\*Any high-grade mineral oil such as Polarine or Mobilol can be used.

allowed to rest on the shoulder at the end of the piston. To opposite sides of this tube, near its upper end, are soldered two projections (see side elevation), on which rest the wooden lever, which is hinged to a block as shown in the illustration. Two wires also connect the handle of the piston with the lever. The syringe must be firmly held in a clamp at its upper end, and cotton or asbestos is placed between the clamp and the glass, so as to prevent breakage and permit of some lateral motion when the lever is manipulated.

The first step in preparing the blood pump is to get rid of all the dead space in the tubing and connections. This is readily accomplished, for *E* by turning stopcock *C* so that *E* communicates with *B*, raising the leveling burette (*G*), and simultaneously withdrawing the piston of the syringe, by depressing the lever, until about 20 mm. of mercury has collected on the top of the piston. The stopcock is then turned so that *B* and *D* are connected and the piston raised until all the air is expelled and mercury completely fills tube *D*. Any drops of mercury falling from the open end of *B* must be caught in a small beaker. The mercury left on the top of the piston seals this completely during the subsequent manipulations.

After squeezing all air out of the tubing on the blood bulb, this is now connected with *D* and immersed in a jug containing water at 45° C. Having turned *C* so that *B* communicates with *D*, the piston is then depressed to about the 20 c.c. mark, and while still depressed the screw clip (1) is opened. About this time the blood will begin to boil and the gases given off from it will pass into the vacuum above the mercury in the syringe. *C* is turned so that *B* is closed off and the piston allowed slowly to ascend. (It must not be allowed to ascend too rapidly, since this might break the syringe.) The gas which has collected in the syringe is now expelled into the burette (*F*) by turning *C* so that *B* and *E* communicate and pressing up the piston. After all the gas is out of the syringe, mercury is allowed to run into *E* a short distance, being careful not to allow any to get into *F*. This first process obviously removes only a small fraction of the total gas in the blood, and it must be repeated several times exactly as described above, until no more gas can be secured. The dislodgment of the gas from the blood is greatly accelerated by warmth and by occasionally removing the bulb from the water-bath and shaking briskly.

It is now necessary to measure and analyze the evolved gas. For this purpose the piston is cautiously pushed up, with *E* and *B* in communication, until mercury stands at the zero mark on the neck of the gas burette. The clip (4) is then screwed down and the leveling burette (*G*) lowered until the meniscus stand at the same level in it and the burette. This brings the gas to atmospheric pressure and the volume is read and noted. The reading gives the c.c. of gas in 10 c.c. blood. The volume should be reduced to standard temperature and pressure (for calculation see p. 431 of this JOURNAL). To analyze the gas a 40 per cent solution of sodium hydroxide is sucked from a watch glass into the 2 c.c. all-glass syringe (*I*), and the tube attached to the nozzle inserted in the side tube (*H*). All air must be expelled from this tube. With the pinchcock (5) closed, the clip (5) is opened, while gentle pressure is being maintained on the piston of the small syringe so that the mercury may not run into it. The NaOH runs up to the top of the mercury column (*I'*), and when it is all in,

clip 5 is again screwed down. The syringe (*I*) is removed and *F* inverted several times so that the carbon dioxide in the gas contained in it may be thoroughly absorbed. On now opening clip 6, the mercury will rise in *F*, and by adjusting the leveling tube the shrinkage in volume due to the absorption of  $\text{CO}_2$  can be ascertained and the percentage of this gas determined. The reading is taken which corresponds to the top of the NaOH solution, a similar amount of NaOH solution being placed on the top of the mercury in the leveling tube. Care must be taken to see that all the  $\text{CO}_2$  is absorbed.

To absorb the oxygen, about a gram of pyrogallic acid is dissolved in 2 c.c. of water in the watch glass, the solution is introduced into *F*, and the further manipulations conducted in the same manner as for the NaOH solution. The gas which remains when both  $\text{CO}_2$  and  $\text{O}_2$  are absorbed is nitrogen. There should not be more than 0.1-0.2 c.c., any larger amount being due to air leakage into the apparatus during the manipulations. If any considerable amount of nitrogen is left, its volume should be measured, and by subtracting 0.2 from this, the volume of  $\text{O}_2$  that must have been introduced, as air, with it can be ascertained and subtracted from the actually observed  $\text{O}_2$ . By taking proper precautions, however, the residual nitrogen should never be more than 0.3-0.5 c.c.

When the analysis is completed, the mercury is run out from the burette by the side tube (*H*), after removing the stopcock (*C*), and the burette thoroughly washed with water. The mercury and alkali pyrogallate solution (which is now brown in color) are then washed in running water until the washings react neutral to litmus paper. The mercury should then be transferred to a separating funnel containing a dilute solution of sulphuric acid. The blood bulb should also be cleaned immediately, since otherwise a sticky precipitate which is difficult to remove adheres to the walls.

---

### SIMPLE METHOD OF MEASURING ANTISHEEP AMBOCEPTOR CONTENT OF HUMAN SERUM AND CORRECTING FOR IT IN WASSERMANN TESTS

---

By J. J. SEELMAN, M.D., MILWAUKEE, WIS.

NATIVE antisheep amboceptors are present in varying amounts in most human bloods. It has long been evident to serologists that this factor introduces a source of error in complement-fixation tests in which the sheep hemolytic system is employed. If the hemolytic system is to perform its function of indicator with precision its action must be uniform for every serum tested in a given test. In the classical Wassermann test the native antisheep amboceptors are ignored. As a result the carefully titrated system of known hemolytic strength becomes a system of unknown strength the moment it is added to the antigen serum-complement mixture. If it is conceded that a carefully titrated hemolytic system is essential to accurate work, it necessarily follows that any factor which tends to throw this system out of adjustment the moment it is about to exercise its function of indicator should, if possible, be removed.

If a human hemolytic system is employed, as in the Noguchi test, the an



sheep amboceptors can, of course, be ignored. Practical considerations have, however, prevented the general adoption of the human hemolytic system. It is much more difficult to immunize rabbits against human corpuscles than against sheep corpuscles. The hemolytic titer for the former can never be brought as high as for the latter, while the agglutinating titer is usually much higher. Isohemolysins as well as isoagglutinins also interfere with the accuracy of this system.

Antisheep amboceptors can be removed by subjecting the serum to the action of sheep corpuscles and subsequently removing the latter by centrifugation. This requires additional handling and repipetting of sera, consumes considerable time and increases the chances of error. There is also unquestionably an osmotic interchange between corpuscles and serum, and just how far this may influence the specific behavior of the serum in the test has not been determined. That serum is considerably more anticomplementary after this treatment than before has been demonstrated.

The titration of each serum to determine its antisheep amboceptor content can, of course, be carried out, but requires an amount of labor inconsistent with the requirements of routine work.

I have for some time used, with complete satisfaction, a simple method which is practically accurate, and requires the setting up of but one tube for each serum to be tested in addition to an amboceptor titration.

Essentially the method consists of utilizing the tubes of an amboceptor titration as standards by which to measure the hemolyzing power of the serum to be examined. The detailed technic must vary somewhat with the methods employed in different laboratories.

In our laboratory we use one-fourth Wassermann quantities. Our amboceptor is dried on filter paper in which condition it keeps its titer unchanged for at least six months. As any one batch of amboceptor is consumed in much less time than this, the originally ascertained titer can always be accepted as correct. The titer of a new amboceptor is established by comparisons with the old, and by titrations with pooled guinea pig serum. The amboceptor is diluted so that the dose (two units) is .14 c.c. and with it as a standard we titrate the complement before each test. Along with this complement titration, the native amboceptor content of each serum to be examined is ascertained as follows:

Two series of tubes are set up. One series is an amboceptor titration, consisting of seven tubes, each receiving .25 c.c. of a 1:10 guinea pig serum dilution, .25 c.c. 5 per cent corpuscle suspension, and increasing amounts of amboceptor dilution, .02, .04, .06, .08, .1, .12 and .14 c.c. respectively. The second series consists of one tube for each serum to be tested, receiving each .25 c.c. complement dilution, .25 c.c. corpuscle suspension and .05 c.c. of its respective, inactivated serum, but no amboceptor. This set-up is placed in the water-bath at 37.5 C. for one-half hour. With an average complement the .14 c.c. amboceptor tube will begin to hemolyze in about five to six minutes and will be completely hemolyzed in about ten to twelve minutes, varying somewhat with different guinea pig sera. When about 75 per cent hemolysis has taken place in the .14 c.c. amboceptor tube it is taken out and compared with each of the serum tubes and the sera of those tubes which correspond to it in degree of hemolysis

are considered as having a value of .14 c.c. amboceptor. The same procedure is carried out with the .12, .1 and .08 c.c. amboceptor tubes when each has attained about 75 per cent hemolysis. At the end of the half-hour the .08 c.c. amboceptor tube is usually completely hemolyzed and the .06 c.c. shows slight inhibition. At this time, without waiting for further hemolysis, the .06, .04 and .02 c.c. amboceptor tubes are compared with the remaining serum tubes and each of these latter is given an amboceptor value corresponding to the amount of amboceptor contained in the amboceptor tube with which it compares. The comparisons, of course, are not always exact, and when they fall between two amboceptor tubes the selection is made between them. For instance, if a serum tube falls between the .06 and .08 c.c. amboceptor tube, it is read as containing .07 c.c. amboceptor.

In the test proper the required allowances are made. If a serum was shown to contain a native amboceptor value corresponding to .14 c.c. (the full dose) of the amboceptor dilution to be used, it receives no amboceptor whatever in the test; if its value is less than this, it receives the difference between its value and .14; if its value is less than .02 c.c. it receives the full dose of amboceptor.

Not infrequently sera are encountered which hemolyze faster than the .14 c.c. amboceptor tube. If the difference is only slight, it is ignored. If it is considered sufficient to act as a source of error, the native amboceptor is removed altogether and the serum given the full dose in the test.

For the purposes of the above described native amboceptor determinations, the complement titer of the guinea pig serum is immaterial, the only condition being that the same amount of guinea pig serum and the same amount of corpuscles are used in the amboceptor standards as are used in the serum unknowns. For this reason, when complement titrations are made before the Wassermann test, the native amboceptor determinations can be made along with the complement titration, and require no additional time except that consumed in setting up the necessary tubes. If no complement but only an amboceptor titration is made, the native amboceptor determinations can be made by the simple addition of the serum tubes, as above described, to the usual amboceptor titration.

Following are the native amboceptor determinations made on 500 sera recently tested in our laboratory with an amboceptor dilution of which the dose (two units) was .14 c.c.:

AMBOCEPTOR VALUE		
Less than .02 c.c.		166
.02 " to .04 c.c.		79
.04 " " .06 "		75
.06 " " .08 "		56
.08 " " .10 "		30
.10 " " .12 "		29
.12 " " .14 "		25
.14 " " .14 " +		19
.14 " " .14 " ++		12
.14 " " .14 " +++		9

That the correction of these wide variations of native antishoop amboceptor content must add considerably to the accuracy of the Wassermann test is self-evident.

# *The Journal of Laboratory and Clinical Medicine*

VOL. III.

JULY, 1918

No. 10

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.

Ann Arbor, Mich.

## ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D.	- - -	ST. LOUIS
HANS ZINSSER, M.D.	- - -	NEW YORK
PAUL G. WOOLLEY, M.D.	- - -	CINCINNATI
FREDERICK P. GAY, M.D.	- - -	BERKELEY, CAL.
J. J. R. MACLEOD, M.B.	- - -	CLEVELAND
ROY G. PEARCE, M.D.	- - -	CLEVELAND
ROGER S. MORRIS, M.D.	- - -	CINCINNATI
GERALD B. WEBB, M.D.	- - -	COLORADO SPRINGS
E. E. SOUTHARD, M.D.	- - -	BOSTON

Contents of this Journal Copyright, 1918, by The C. V. Mosby Company—All Rights Reserved  
Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

## *EDITORIALS*

### *Substitutes for Blood in Transfusion*

THERE exists, as Rous and Wilson<sup>1</sup> say, a great and urgent need for an injection fluid that can be satisfactorily employed instead of blood for transfusion in cases of hemorrhage. That this need is greatest at or near the battle fronts in Europe goes without saying. Nevertheless there is a constant need of such a fluid in everyday emergency work at home.

The reason that blood is the ideal transfusion fluid is not that it is blood nor that it contains hemoglobin or other blood substances, but because the liquid (the water) that it contains is held by the colloids of the fluid in such a way that it is held by them for a longer period than so much pure water would be and therefore it remains longer in the vessels and gives the heart something to push against, so to speak. If one introduces pure water into the vessels of the body it is almost immediately given up and excreted by the kidneys. Water held by colloids, on the contrary, tends to be held in the body until the colloids holding the water are split up by the ferments of the body and the water is set free. Salt solutions not held in colloidal combination act as does pure water. Obviously the ideal fluid for injection after hemorrhage is blood plasma, for it is a colloidal solution in which the water is held in a physiologic stable way. But, also ob-

viously plasma is not always obtainable on short notice. It has been shown that when more than half the total calculated blood volume had been taken from an animal and when the carotid pressure had fallen to the physiologic zero, the pressure was instantly and permanently restored to normal by injecting an equivalent amount of plasma. A saline solution on the other hand brought about only a slight transient recovery of the pressure.

As substitutes for plasma or blood in transfusion Hogan<sup>2</sup> has recommended and used a 2.5 gelatin solution, and Bayliss<sup>3</sup> a 6 per cent gelatin or a 7 per cent gum acacia solution. The acacia has the advantage over gelatin that it can be sterilized without danger of hydrolyzing it and thereby rendering it useless. Boiling it does no damage. Also acacia is protein-free and therefore will not produce anaphylaxis. A 2 per cent acacia solution at first raises the lowered blood pressure to normal but the rise is very transient. A 4 per cent solution is more satisfactory, but this, or even a 5 per cent solution, is not effective in all cases. Six or 7 per cent is required if one is to bring back the normal pressure in an organism depleted of its fluid reserves.

As Rous and Wilson say, the needs for a blood substitute may be widely different in different cases. When the hemorrhage has been rapid and has been completely checked, almost any harmless isotonic solution will tide the patient over. It matters little that the fluid will soon leave the vessels, for the patient's fluid reserves are almost intact, as is his ability to manufacture a plasma rapidly. At the other extreme are those instances in which the blood has been draining steadily away and there remains in the body no source of an immediate restoration of fluid. Here half-measures can not suffice. A fluid must be furnished which will take the place, over many hours, of the lost blood bulk. Except for the blood or plasma of other human beings, fluids containing from 6 to 7 per cent of gum acacia are the best at present available for the purpose. Intermediate cases can undoubtedly be much helped by a 2 or 3 per cent acacia solution or by Hogan's solution. In view of our ignorance of the after-effects of these foreign substances it is advisable not to inject more than the needs of the case demand.

#### BIBLIOGRAPHY

<sup>1</sup>Rous and Wilson: Jour. Am. Med. Assn., 1918, lxx, 219.

<sup>2</sup>Hogan: Ibid., 1915, lxvii, 721.

<sup>3</sup>Bayliss: Proc. Roy. Soc., lxxxix, 380.

—P. G. W.

### *The Duty of the Employer in the Reconstruction of the Crippled Soldier*

WE must count on the return from the front of thousands of crippled soldiers. We must plan to give them on their return the best possible chance for the future.

Dependence cannot be placed on monetary compensation in the form of a pension, for in the past the pension system has proved a distinct failure in so far as constructive ends are involved. The pension has never been enough



to support in decency the average disabled soldier, but it has been just large enough to act as an incentive to idleness and semi-dependence on relatives or friends.

The only compensation of real value for physical disability is rehabilitation for self-support. Make a man again capable of earning his own living and the chief burden of his handicap drops away. Occupation is, further, the only means for making him happy and contented.

Soon after the outbreak of hostilities the European countries began the establishment of vocational training schools for the rehabilitation of disabled soldiers. They had both the humanitarian aim of restoring crippled men to the greatest possible degree and the economic aim of sparing the community the burden of unproductivity on the part of thousands of its best citizens. The movement had its inception with Mayor Edouard Herriot of the city of Lyons, France, who found it difficult to reconcile the desperate need for labor in the factories and munition works while men who had lost an arm or a leg but were otherwise strong and well were idling their time in the public squares. He therefore induced the municipal council to open an industrial school for war cripples which has proved the example and inspiration for hundreds of similar schools since founded throughout France, Italy, Germany, Great Britain and Canada.

The disability of some crippled soldiers is no bar to returning to their former trade, but the injuries of many disqualify them from pursuing again their past occupation. The schools of training prepare these men for some work in which their physical handicap will not materially interfere with their production.

The education of the adult is made up largely of his working experience. The groundwork of training in his past occupation must under no circumstances be abandoned. The new trade must be related to the former one or be, perhaps, an extension or specialization of it. For example, a man who had done manual work in the building trades may by instruction in architectural drafting and the interpretation of plans be fitted for a foreman's job, in which the lack of an arm would not prove of serious handicap. A trainman who had lost a leg might wisely be prepared as a telegrapher, so that he could go back to railroad work, with the practice of which he is already familiar.

Whatever training is given must be thorough, for an adult can not be sent out to employment on the same basis as a boy apprentice. He must be adequately prepared for the work he is to undertake.

The one-armed soldier is equipped with working appliances which have supplanted the old familiar artificial limb. The new appliances are designed with a practical aim only in view; they vary according to the trade in which the individual is to engage. For example, the appliance for a machinist would be quite different from that with which a wood turner would be provided. Some appliances have attached to the stump a chuck in which various tools or hooks can interchangeably be held. The wearer uses these devices only while at work; for evenings and holidays he is provided with a "dress arm" which is made in imitation of the lost natural member.

An important factor in the success of re-educational work is an early start, so that the disabled man shall have no chance to go out unemployed into the community. In even a short period of exposure to the sentimental sympathy of family and friends, his "will to work" is so broken down that it becomes difficult again to restore him to a stand of independence and ambition. For this reason, therefore, the plan for his future is made at as early a date as his physical condition admits, and training is actually under way before the patient is out of the hospital.

In the readjustment of the crippled soldier to civilian life, his placement in employment is a matter of the greatest moment. In this field the employer has a very definite responsibility.

But the employer's duty is not entirely obvious. It is, on the contrary, almost diametrically opposite to what one might superficially infer it to be. The duty is not to "take care of" from patriotic motives, a given number of disabled men, finding for them any odd jobs which are available, and putting the ex-soldiers in them without much regard to whether they can earn the wages paid or not.

Yet this method is all too common. A local committee of employers will deliberate about as follows: "Here are a dozen crippled soldiers for whom we must find jobs. Jones, you have a large factory; you should be able to take care of six of them. Brown, can you not find places for four of them in your warehouse? And Smith, you ought to place at least a couple in your store."

Such a procedure can not have other than pernicious results. In the first years of war the spirit of patriotism runs high, but experience has shown that men placed on this basis alone find themselves out of a job after the war has been over several years, or in fact, after it has been in progress for a considerable period of time.

A second weakness in this method is that a man who is patronized by giving him a charity job, comes to expect as a right such semi-gratuitous support. Such a situation breaks down rather than builds up character, and makes the man progressively a weaker rather than a stronger member of the community. We must not do our returned men such injury.

The third difficulty is that such a system does not take into account the man's future. Casual placement means employment either in a makeshift job as watchman or elevator operator such as we should certainly not offer our disabled men except as a last resort—or in a job beyond the man, one in which, on the cold-blooded considerations of product and wages, he can not hold his own. Jobs of the first type have for the worker a future of monotony and discouragement. Jobs of the second type are frequently disastrous, for in them a man, instead of becoming steadily more competent and building up confidence in himself, stands still as regards improvement and loses confidence every day. When he is dropped or goes to some other employment, the job will have had for him no permanent benefit.

Twelve men sent to twelve jobs may all be seriously misplaced, while the same twelve placed with thought and wisdom and differently assigned to the same twelve jobs may be ideally located. If normal workers require expert

and careful placement, crippled candidates for employment require it even more.

The positive aspect of the employer's duty is to find for the disabled man a constructive job which he can hold on the basis of competency alone. In such a job he can be self-respecting, be happy, and look forward to a future. This is the definite patriotic duty. It is not so easy of execution as telling a superintendent to take care of four men, but there is infinitely more satisfaction to the employer in the results, and infinitely greater advantage to the employee. And it is entirely practical, even in dealing with seriously disabled men.

A cripple is only debarred by his disability from performing certain operations. In the operations which he can perform, the disabled man will be



BACK TO THE OLD JOB.

Most of our wounded will be able to resume their former occupations. Although he lost both legs, this soldier will continue in his profession as a draftsman. At present he is being physically reconstructed at Walter Reed Hospital, Washington, D. C., but as soon as his artificial limbs are fitted he will leave. Meanwhile, the most effective curative agent is his occupation with mechanical drafting. (Courtesy "Carry On.")

just as efficient as his non-handicapped colleague, or more so. In the multiplicity of modern industrial processes it is entirely possible to find jobs not requiring the operations from which any given type of cripples are debarred. For such jobs as they can fill the cripple should be given preference.

Thousands of cripples are now holding important jobs in the industrial world. But they are men of exceptional character and initiative and have, in general, made their way in spite of employers rather than because of them. Too many employers are ready to give the cripple alms, but not willing to ex-

pend the thought necessary to place him in a suitable job. This attitude has helped to make many cripples dependent. With our new responsibilities to the men disabled in fighting for us, the point of view must certainly be changed. What some cripples have done, other cripples can do—if only given an even chance.

The industrial cripple should be considered as well as the military cripple, for in these days of national demand for the greatest possible output there should not be left idle any men who can be made into productive workers.

With thoughtful placement effort, many men can be employed directly on the basis of their past experience. With the disabled soldiers who profit by the training facilities the government will provide, the task should be even easier.

This, then, constitutes the charge of patriotic duty upon the employer:

To study the jobs under his jurisdiction to determine what ones might be satisfactorily held by cripples. To give the cripples preference for these jobs. To consider thoughtfully the applications of disabled men for employment, bearing in mind the importance of utilizing to as great an extent as possible labor which would otherwise be unproductive. To do the returned soldier the honor of offering him real employment, rather than proffering him the ignominy of a charity job.

If the employer will do this, it will be a great factor in making the complete elimination of the dependent cripple a real and inspiring possibility.

DOUGLAS C. McMURTRIE, DIRECTOR,  
*Red Cross Institute for Crippled and Disabled Men,*  
*New York City.*



# *The Journal of Laboratory and Clinical Medicine*

VOL. III.

ST. LOUIS, AUGUST, 1918

No. 11

## ORIGINAL ARTICLES

### COMMUNICABLE DISEASES IN THE NATIONAL GUARD AND NATIONAL ARMY OF THE UNITED STATES DURING THE SIX MONTHS FROM SEPTEMBER 29, 1917, TO MARCH 29, 1918\*

BY COL. VICTOR C. VAUGHAN, M.C., N.A., AND CAPT. GEORGE T. PALMER, S.C.  
N.A., WASHINGTON, D. C.

#### PREFACE

THE authors would call attention to the fact that this report has been prepared with the object of giving publicity during the present year to the salient features of communicable disease incidence in the United States Army during the past winter. The statistical data have been drawn from the telegraphic reports sent in weekly from each camp, cantonment and army post or station. These reports are altered somewhat later on due to change of diagnosis or some other cause and the results here given may not agree in every detail with the more permanent and complete personal histories sent in to the Sick and Wounded Division each month. Delay in reporting makes the latter data unavailable for current use.

#### INTRODUCTION

The purpose of this report is to take stock of health matters in our armies, to study the data furnished in weekly telegraphic reports, to review the records of sanitary inspectors and epidemiologists, to ascertain what diseases have appeared in the armies and the extent of their spread, and to investigate the avenues through which these infections have found their way into the camps. The mobilization of raw, untrained men and their hurried transformation into

\*A Report from the Section of Communicable Diseases, Division of Sanitation, prepared under the direction of Colonel Deane C. Howard, and Published by Permission of the Surgeon General.

effective soldiers, has always been accompanied by marked increase in morbidity and mortality. The assembly of young men in camps acts like a dragnet bringing to a central point all infections prevalent in the area from which these men came. The wider the area, the larger the numbers of those brought together, the greater the susceptibility of the individuals constituting the assembly, the more closely these individuals are crowded together and the more intimate their contact, the larger the number of bearers of the infections, the more virulent the disease-causing organisms brought into the camps, the greater will be the morbidity and mortality from communicable diseases. Our government has assembled within less than one year more than one million untrained, undisciplined men, the most of whom were quite ignorant of personal hygiene and without previous experience in caring for themselves under conditions of army life. That the morbidity and mortality from communicable diseases among these should show an average above the figures shown in the civilian life from which they came, was to be expected by one familiar with the science of epidemiology.

We have chosen to cover the time from September 29, 1917, to March 29, 1918, because the camps were fairly well developed on the first of these dates and the period covers the winter months and our findings can be compared with the summer months when there will be felt the seasonal influence on the character and spread of infections. Furthermore, lessons learned from these studies may give information which possibly may be utilized with great advantage next winter.

This report covers and is confined to the time period mentioned and any conclusions that are drawn may be considered tentative and founded upon the evidence so far secured and subject to such modification as may be justified by additional information.

The data that follow deal for the most part with thirteen National Guard Camps and sixteen National Army Cantonments, which represent over 70 per cent of the troops in this country during the period in question. Records from small and scattered units and from the various posts of the Regular Army are omitted, as the data from these places are less readily subjected to statistical analysis. All of these facts will, of course, be included in later reports. The data from four large guard camps which were in existence in October are omitted because the men were shifted elsewhere shortly afterward. The present report deals with camps which were in existence continuously throughout the six months.

#### COMPARATIVE MORTALITY IN ARMY AND CIVIL LIFE

In studying this phase of our problem there are several facts which must be considered. Some of the more important of these are as follows:

The death rate in the Army should be compared with that for the same age period in civil life. The comparison should be made on the records for the same time of the year and so far as possible for the same year. Through the courtesy of the health and vital statistics departments of certain cities we are able to do this. The greater number of enlisted men in the army are between 21 and 31 years of age. The period nearest this available in civil statistics is

the age from 20 to 29 years. In all comparisons between military and civilian death rates the following should be borne in mind:

(1) The death rate of the group 20 to 29 years is lower than that of the present draft age 21 to 31 years.

(2) The death rate in these age groups is greater among males than among females.

(3) The army includes more above than below the draft age. In all these respects a comparison is slightly to the disadvantage of the army.

The figures for this comparison are expressed as annual rates calculated on actual deaths occurring during the period.

TABLE 1

## ANNUAL DEATH RATE PER 1000

(Age 20 to 29 Years, Time, Oct., Nov., Dec., 1917; Jan., Feb., Mar., 1918.)

PLACE	DEATH RATE
Army	9.1
New York City	5.5
Saint Louis	5.5
New Orleans	10.4
Pittsburg	6.2
Chicago	5.2

To obtain this information, requests were sent the Commissioners of Health of all states and a number of cities within the Registration Area. It is on the replies received that the civil statistics of the past winter are based. There are instances of wide variation in the rates of certain diseases. The accuracy of some of these may be questioned. Errors may have been caused by improper reporting or else by erroneous population figures used in the computation of rates. Populations have been estimated in the customary manner followed by the Census Bureau which assumes increases in arithmetical progression. This is open to error because the present is the eighth year since the last census of 1910, and we do not know that populations in all cases have increased in this manner.

It is seen from these figures that the average death rate for the whole army is higher than that of any city in the list with the exception of New Orleans.

The distribution of death rates by age groups in the Registration Area of the United States during the winter months of 1915, is shown by the census of that year in Table 2, in comparison with the army rate during corresponding months of 1917-1918.

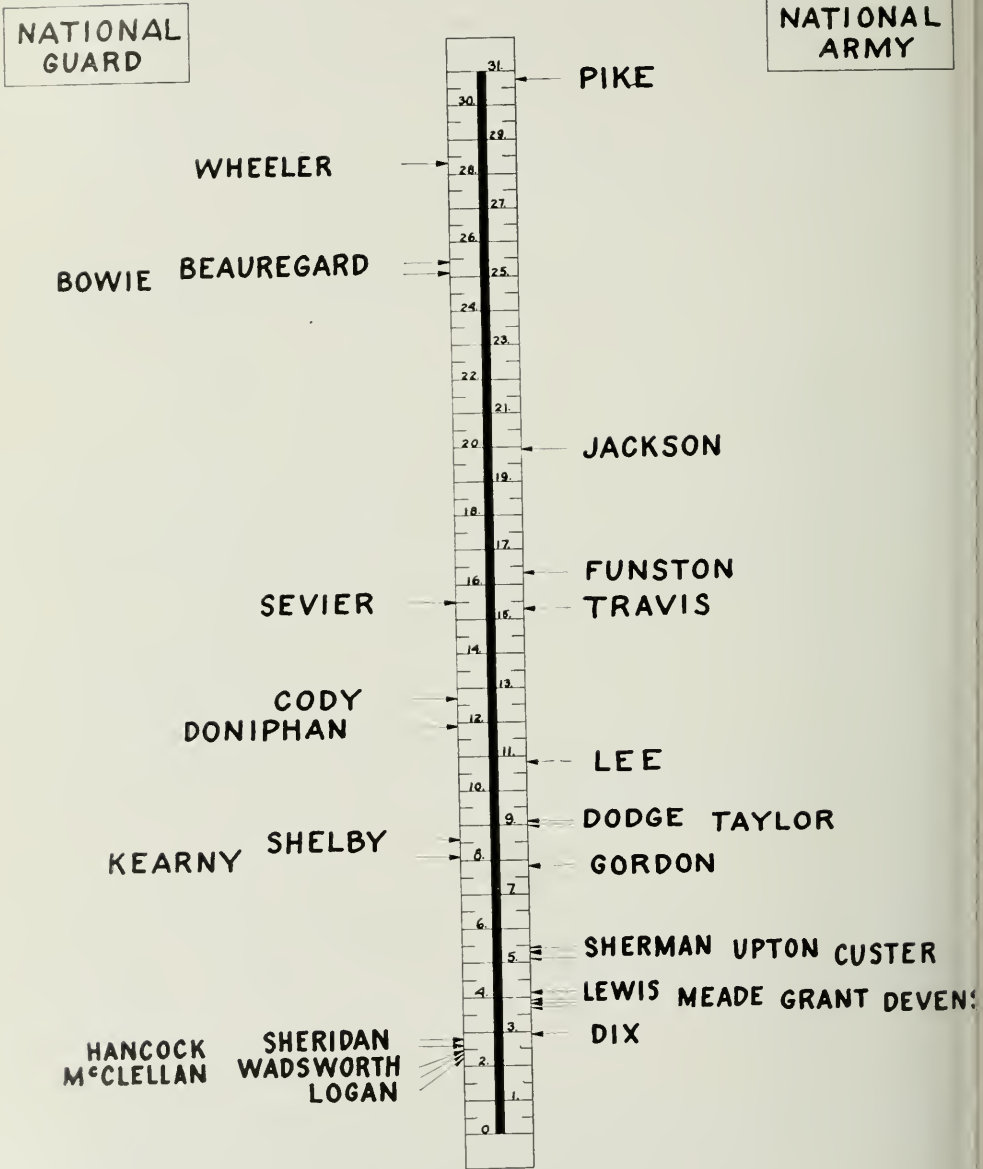
TABLE 2

PLACE	DEATH RATE PER 1000
Army	9.1
U. S. Registration Area:	
All ages	14.3
Age 20 to 49	8.3
Age 20 to 39	6.8
Age 20 to 29	5.7

The Registration Area in 1915 included 25 states, the District of Columbia, and 41 cities in non-registration states. Included among the registration states is North Carolina, the returns from which relate only to municipalities having 1000 or more inhabitants in 1910. The states and cities making up this area are those for which the registration of deaths has been accepted as being approximately complete (at least 90 per cent of all deaths being registered) and which were admitted to the Registration Area only after it was known from the data at hand that the deaths were being recorded properly under State law or for cities, under municipal ordinance.

These figures show that while the older age groups have the higher rates, all below 49 are lower than the Army. It must be borne in mind, however, that the Registration Area covers a wide diversity in density of population, while the closeness of contact in the camps is greater and more continuous than that

# DEATHS FROM ALL CAUSES IN ARMY CAMPS



ANNUAL DEATH RATE PER 1000  
SEPT. 29, 1917 TO MAR. 29, 1918

Chart I.



of the most crowded city. Furthermore, the Registration Area does not include the States of South Carolina, Georgia, Florida, Tennessee, Alabama, Mississippi, Louisiana, Oklahoma and Texas, where death rates are higher. As will be seen in Table 3, which gives the rates for each camp, there are 13 camps out of 29 with rates below the rate for age group 20 to 29 in the Registration Area. It may be pointed out also that while some deaths may escape report in civil life, all in the camps are reported.

It is a striking fact that the death rates in the different camps show wide variation as is indicated in Table 3.

TABLE 3  
(See Chart 1)  
ANNUAL DEATH RATE PER 1000

NATIONAL GUARD		NATIONAL ARMY	
Wheeler	28.3	Pike	30.7
Beauregard	25.4	Jackson	19.9
Bowie	23.1	Funston	16.3
Sevier	15.5	Travis	15.3
Cody	12.7	Lee	10.8
Doniphan	11.9	Dodge	9.1
Shelby	8.6	Taylor	9.0
Kearny	8.1	Gordon	7.8
Sheridan	2.8	Sherman	5.4
Hancock	2.6	Upton	5.3
Wadsworth	2.5	Custer	5.1
McClellan	2.4	Lewis	4.1
Logan	2.3	Meade	3.9
		Grant	3.8
		Devens	3.7
		Dix	2.9

It will be seen by comparing Table 3 with Table 1 that five National Guard camps (Sheridan, Hancock, Wadsworth, McClellan and Logan) have shown lower death rates than New York City, St. Louis, New Orleans, Pittsburg and Chicago for the age group 20 to 29 years. Two more National Guard camps (Shelby and Kearney) show lower death rates than New Orleans for this age group. Six National Army camps (Custer, Lewis, Meade, Grant, Devens and Dix) show lower death rates than any of the above mentioned cities. Two other National Army camps (Sherman and Upton) are about on a level with New York, St. Louis and Chicago, and below Pittsburg and New Orleans. Three more National Army camps (Dodge, Taylor and Gordon) are below New Orleans. These facts demonstrate that camp life may be made as safe as that of some of our best health-guarded cities, and indeed may show a lower death rate in the corresponding age group. It is worthy of note that Camps Wadsworth and Upton, both made up largely of New York troops, compare favorably with New York City; that Hancock, composed of the Pennsylvania National Guard, is better than Pittsburg; that Logan, occupied by the National Guard of Illinois, is better than Chicago; that Doniphan, occupied by the Missouri National Guard, is worse than St. Louis; and that Beauregard, the location of the National Guards of Louisiana, Mississippi and Arkansas, is far worse than New Orleans. Chart II is presented to show comparative death rates in civil life and National Guard and National Army Camps.

In Table 4 are given the site of the camps and States which furnished troops to each camp.

The question of the wide variations in the death rates in the different camps is a difficult one to answer. There are evidently many factors involved, some of which will be briefly discussed.

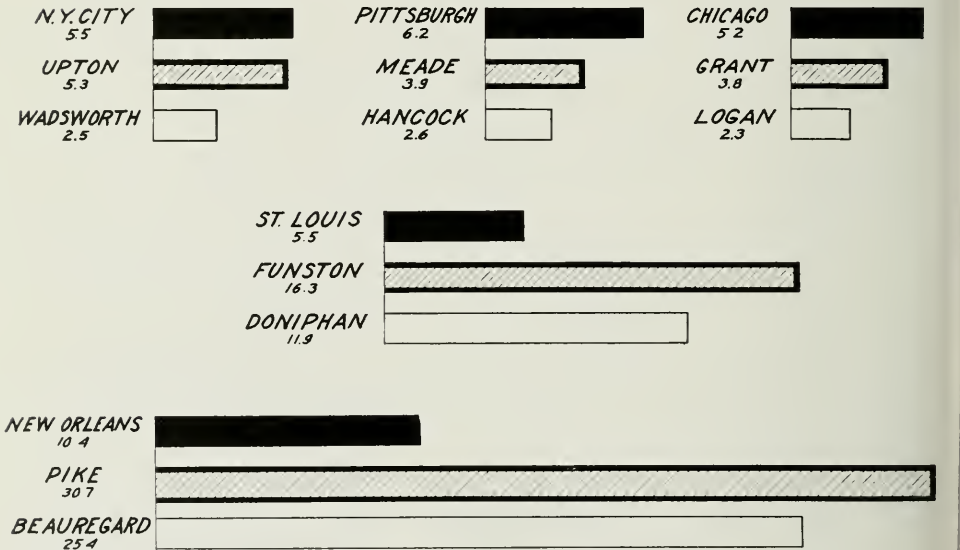
(1) Some of the camps have acted as filters through which many troops have passed, leaving their most unfit on the filter. To figure the average strength of these camps as a basis for the computation of their death rate is hardly fair, but we know of no other way to do this. It is a striking thing that the worst camps as measured by their death rates are bad when measured by sick admissions, noneffective rate, and prevalence of each of the most important communicable diseases, including venereal disease, the last of which certainly is not

### COMPARATIVE MORTALITY IN CAMP AND CIVIL LIFE

CIVIL LIFE REPRESENTED BY AGE GROUP 20-29 YEARS

ANNUAL DEATH RATE PER 1000

6 MOS. PERIOD SEPT. 29,17 TO MAR 29,18



NOTE THE ABOVE CAMPS CONTAIN MEN RECRUITED FROM THE GENERAL VICINITY OF THE CITY WITH WHICH THEY ARE COMPARED THE UPPER CAMPS ARE NATIONAL ARMY THE LOWER NATIONAL GUARD.

Chart II.

influenced by weather, housing, clothing, camp sanitation, medical or health supervision of the camp. This is graphically shown by Chart III. In a general way it can be stated that those camps which may be designated as closed camps and which have been least employed as filters are the healthiest camps. However, there are exceptions to this.

These filter camps may suffer so far as morbidity and mortality rates are concerned in two ways. First, the camps from which troops have come may have selected the least desirable men for transfer. There are reasons for suspecting that this has been done. In this way the filter camp suffers on account of the inferiority of the material received. In the second place when the troops pass on to another camp many of the unfit may be left behind. Funston and Pike have been notable filter camps as thus explained.

Wadsworth may be taken as an example of a closed camp. Practically no

TABLE 4

LOCATION OF NATIONAL GUARD AND NATIONAL ARMY CAMPS, TOGETHER WITH THE STATES FROM WHICH MEN ARE DRAWN

OCTOBER, 1917, TO MARCH, 1918

*NATIONAL GUARD*

CAMP	SITE	SOURCE OF TROOPS
Beauregard	Alexandria, La.	Arkansas, Louisiana, Mississippi
Bowie	Ft. Worth, Tex.	Oklahoma, Texas
Cody	Deming, N. M.	Iowa, Minnesota, Nebraska, South Dakota
Doniphan	Ft. Sill, Okla.	Kansas, Missouri
Hancock	Augusta, Ga.	Pennsylvania
Kearny	Linda Vista, Cal.	Arizona, California, Colorado, New Mexico, Utah
Logan	Houston, Tex.	Illinois
McClellan	Anniston, Ala.	Delaware, District of Columbia, Maryland, New Jersey, Virginia
Sevier	Greenville, S. C.	North Carolina, South Carolina, Tennessee
Shelby	Hattiesburg, Miss.	Indiana, Kentucky, West Virginia
Sheridan	Montgomery, Ala.	Ohio
Wadsworth	Spartanburg, S. C.	New York
Wheeler	Macon, Ga.	Alabama, Florida, Georgia

*NATIONAL ARMY*

Custer	Battle Creek, Mich.	Michigan, Wisconsin
Devens	Ayer, Mass.	Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, Connecticut, New York
Dix	Wrightstown, N. J.	Delaware, New Jersey, New York
Dodge	Des Moines, Iowa	Illinois, Iowa, Minnesota, North Dakota
Funston	Ft. Riley, Kan.	Arizona, Colorado, Kansas, Missouri, Nebraska, New Mexico, South Dakota
*Gordon	Atlanta, Ga.	Alabama, Georgia, Tennessee
Grant	Rockford, Ill.	Illinois, Wisconsin
Jackson	Columbia, S. C.	Florida, North Carolina, South Carolina
Lee	Petersburg, Va.	Pennsylvania, Virginia, West Virginia
Lewis	American Lake, Wash.	Alaska, California, Idaho, Montana, Nevada, Oregon, Utah, Washington, Wyoming
Meade	Annapolis Junction, Md.	District of Columbia, Maryland, Pennsylvania
Pike	Little Rock, Ark.	Alabama, Arkansas, Louisiana, Mississippi
Sherman	Chillicothe, Ohio	Ohio
Taylor	Louisville, Ky.	Illinois, Indiana, Kentucky
Travis	Ft. Sam Houston, Tex.	Oklahoma, Texas
Upton	Yaphank, L. I., N. Y.	New York

NOTE: The States indicated represent the chief source of troops at each place. There are small increments from other points in a number of camps.

\*This camp was occupied by the troops indicated less than two months, when these troops were sent to Wheeler and replaced at Gordon by draft men from many states.

new troops reached this camp during the period covered by this report. In fact the first accessions came in March and consisted of 1500 select men from Camp Taylor. These men had been in service but a short time and were from the mountains of Kentucky and Tennessee. They came with and continued to develop pneumonia, meningitis and minor diseases and soon had a noneffective rate of 79 per 1000 as compared with 24 for the 27th Division. Before this accession the 27th Division consisted wholly of the New York National Guard and the men came from the larger cities of that State with but few from rural communities.

It is a frequent observation in the reports of the health of organizations that all accessions of unseasoned men are followed by a rise in the morbidity curve of "all diseases," and especially of the acute respiratory diseases.\* On

\*Throughout this paper we use the words "respiratory diseases," in referring to those diseases transmitted through the respiratory organs.

# COMPARATIVE MORTALITY AND MORBIDITY AT NATIONAL GUARD AND NATIONAL ARMY CAMPS FOR 6 MONTHS PERIOD SEPT. 29, '17 - MAR. 29, '18.

Plotted on basis of 100 for highest rate in each cause

1 Deaths All Causes

2 Admissions

3 Deaths from Tb

4 Deaths from Diphtheria

Order of Columns

Morbidity from -

5 Pneumonia

6 Measles

7 Meningitis

8 Scarlet Fever.

9 Typhoid and Paratyphoid.

10. Malaria

11 Venereal Disease.

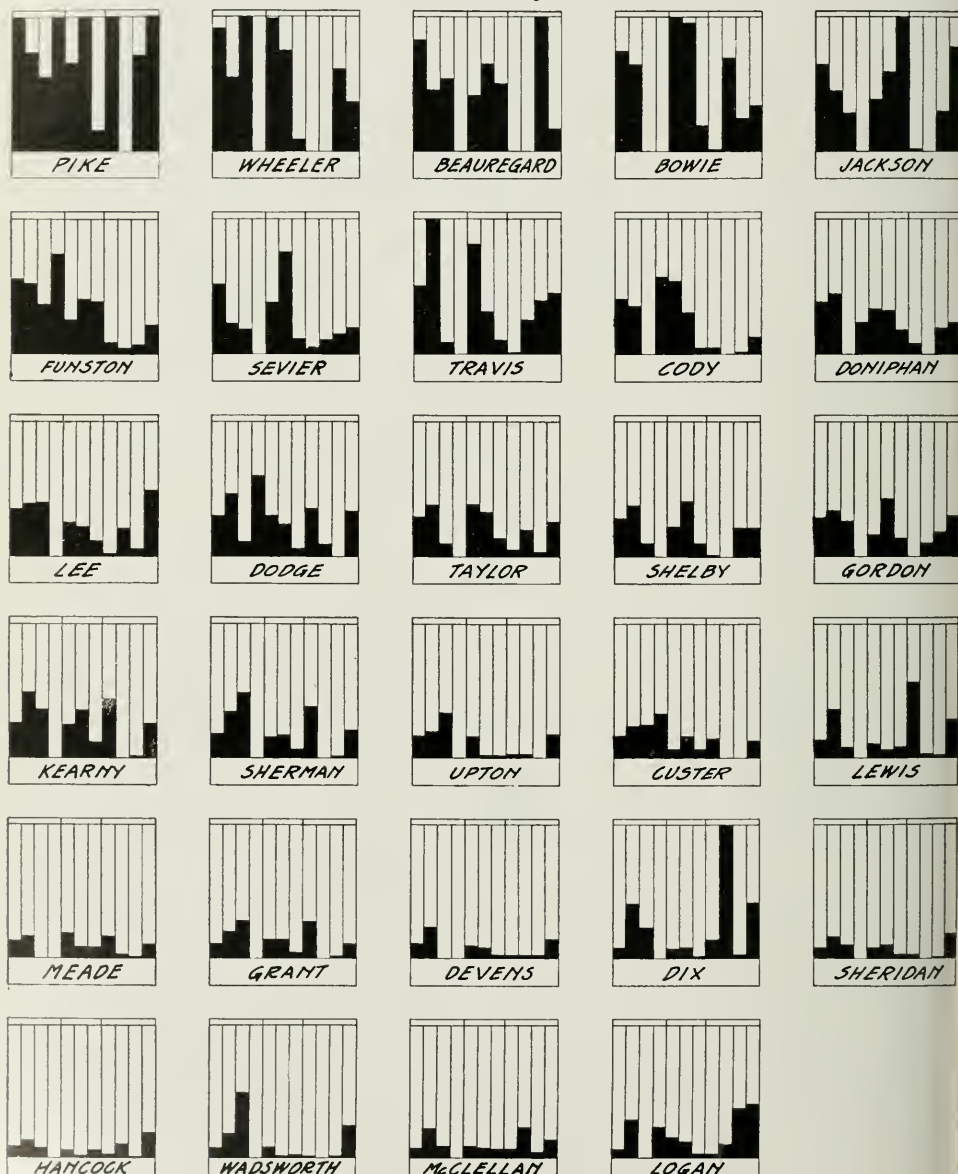


Chart III.



the other hand it seems true that variations in the morbidity curve are less apparent in the more stable organizations. Furthermore, it is frequently stated that the type of an existing disease changes simultaneously with the accession. Lobar pneumonia was the prevalent form of this disease at Grant until troops from Jefferson Barracks arrived, after which bronchopneumonia with streptococcus infection became the predominant type. Fatigue from a long journey by rail may have some effect in lowering resistance, though possibly overcrowding in trains may be a greater factor.

(2) We have tried to ascertain the influence of race, nationality, section of the country from which the troops have come and other similar circumstances on the morbidity and mortality of the several camps, but the data that we have obtained on these points do not justify us in speaking other than tentatively concerning these matters. However, the following points seem to be fairly clear. Under similar conditions the negro is more susceptible to the acute respiratory diseases than the northern white man. The death rate from pneumonia among the negro troops at camp Dodge has been to that from the same disease among the whites as 4.4 is to 1. It will be interesting to note whether this holds true during the warmer months. Southern whites are more susceptible to this disease than are northern whites. These observations are in accord with those of other observers both in this country and abroad. The elder Flint, who practiced medicine both in the North and in the South, made statements to this effect many years ago. The French have found their African troops especially susceptible to pneumonia and have been compelled to provide warmer quarters and heavier clothing for them in the winter than are necessary for the native French soldier. General Gorgas had abundant opportunity to study pneumonia on the Canal Zone and in South Africa, and before the assembly of our troops began he predicted that the acute respiratory diseases and especially pneumonia would be among the most prevalent diseases.

It is an opinion generally held by medical officers in southern camps that hookworm disease and chronic malarial infection increase susceptibility to the acute respiratory diseases. The information we have on these points is not as full as desirable. There has been a careful examination of the feces of the 36th Division at Bowie, with the following results:

Total strength	25,224
Number examined	23,659
Positive hookworm	2,921 = 12.3%
Tapeworm	480 = 2%
Miscellaneous parasites	24

(3) There is no reason for believing that either morbidity or mortality in any camp has been due to faulty sanitation, as we usually understand this term. All the camps are kept clean, have unquestioned water supplies, satisfactory garbage and sewage removal, etc.

The unsanitary camp of 1898, as seen as Chickamauga, and in other encampments of that time, does not exist in the United States today. There undoubtedly has been some faulty handling of the communicable diseases. In certain camps sick men have been allowed to remain in quarters too long for their own good and for the protection of their comrades from infection. There have been mistakes in diagnosis which have favored the spread of infection. In

some camps there has been no attempt to distinguish between the two diseases known under the name of measles and these have been mingled in the hospitals with the result that the patient has had both diseases before he was through. The differential diagnosis between measles and scarlet fever has not always been correct and there has been some mixing of patients in wards devoted to these diseases. Such mistakes occur in private practice, probably with greater frequency, but the consequences are not so serious as they are in the large and crowded army hospitals.

## DEATHS FROM RESPIRATORY DISEASE IN CIVILIAN AND ARMY LIFE

*INCLUDED IN RESPIRATORY GROUP— PNEUMONIA, MENINGITIS, MEASLES, SCARLET FEVER, DIPHTHERIA, TUBERCULOSIS*



### CIVILIAN LIFE

*U.S. REGISTRATION AREA. 6 WINTER MOS. 1915.  
AGE 20 TO 29 YEARS INCL.*



### ALL TROOPS IN U.S.

*6 MOS. SEPT 29, 1917 TO MAR. 29, 1918*

Chart IV.

### COMPARATIVE DEATH RATES IN NATIONAL GUARD AND NATIONAL ARMY

This comparison can not be made in a satisfactory manner, because the two services have been mixed. The National Guard has been in tents and the Army for the most part in barracks, but in some of the National Guard camps quite half the troops have been select or drafted men. The comparison is, therefore, largely between tent and barrack camps. The additions made to the Regular Army have been in tents. With this understanding the death rates in the three organizations are as follows:

(ANNUAL RATE PER 1000)

National Army	9.6
National Guard	9.5
Regular Army	7.8

Comparing the National Army and the Guard, it will appear that the death rates have been practically the same among soldiers in tents and those in barracks. The lower death rate in the new additions to the Regular Army is believed

to be due to the fact that the average age of the men is greater than in the other organizations, and it is furthermore probable that many of these men have had previous service in the Regular Army. Some figures have been secured at Wheeler which point most consistently to the conclusion that men with previous military training show less sick than new men.

We might make comparison between the National Guards of certain States and the National Army from the same States. This is done in Table 5.

TABLE 5  
COMPARISON OF DISEASE INCIDENCE AMONG GUARDSMEN AND NATIONAL ARMY TROOPS FROM  
SIMILAR DISTRICTS

ANNUAL RATE PER 1000	CAMP AND HOME STATE OF TROOPS					
	New York		Ohio		Illinois	
	N. G. Wadsworth	N. A. Upton	N. G. Sheridan	N. A. Sherman	N. G. Logan	N. A. Grant
Deaths, All Causes	2.5	5.3	2.8	5.4	2.3	3.8
<i>Morbidity</i>						
Pneumonia	9.1	15.0	9.3	15	16	14
Meningitis	.8	.5	1	1.7	.7	1.3
Measles	7.9	6.3	41	65	51	58
Scarlet fever	.8	1.5	1.8	17.3	1	12.3
Venereal disease	77.6	57.2	14	67	132	36.4
*Admission, all causes	14.9	16.0	59.8	28	24.3	17.1

\*Average Weekly Admission Rate.

It will be seen from these figures that the death rate in the National Army has, in each case, been higher than in the National Guard. This is probably due to the fact that the National Guard contained more seasoned troops than the National Army. The National Guard Divisions represented in this table were all on the Mexican border in 1916, but were to a variable extent recruited before being assembled in 1917.

#### CAUSES OF DEATH IN THE ARMY

The diseases which have been responsible for the greatest number of deaths in the army during the period covered by this report are the acute respiratory diseases. These may be named in the order in which they have caused deaths, as follows: pneumonia, meningitis, measles, scarlet fever and diphtheria. With the addition of tuberculosis these have caused seventy-seven (77) per cent of all deaths. Sixteen (16) per cent of deaths have been due to other diseases, and

TABLE 6  
(See Chart V)

#### CAUSES OF DEATH IN THE ARMY (ALL TROOPS IN THE U. S.) SIX WINTER MONTHS

CAUSE	PER CENT OF TOTAL DEATHS
Pneumonia	61.5
Other diseases than here mentioned	15.3
Meningitis (all kinds)	12.0
External causes	7.1
Tuberculosis	1.7
Measles	1.1
Scarlet fever	.75
Diphtheria	.46
Typhoid fever	.14

seven (7) per cent due to causes other than disease, such as mechanical injuries. (See Chart IV.)

The per cent of total deaths due to each of the more important causes are shown in Table 6.

The acute respiratory diseases have caused an excessive death rate in the army compared with that due to the same causes in civil life. For the age group from 20 to 29 years, in the registration area in the United States for the five years, 1911 to 1915, deaths from the respiratory diseases, including tuberculosis, amounted to forty-three (43) per cent of total deaths.

TABLE 7  
ANNUAL DEATH RATE PER 100,000

	PNEUMONIA, ALL FORMS	MENIN- GITIS	MEASLES	SCARLET FEVER	DIPH- THERIA	TUBERCULOSIS ALL FORMS
Army	559	109	9.4	6.9	4.2	15.3
U. S. Reg., Area	46	2.4	0.5	1.1	1.8	197
Massachusetts	41	2.3	0.3	1.7	1.4	177
Virginia	111	9.1	2.1	0.5	1.1	602

NOTE: The deaths for civilian life are computed for winter months of 1915.

## CAUSES OF DEATH AMONG ALL TROOPS. U. S. ARMY.

PNEUMONIA 61.5%	
OTHER DISEASES 15.3%	
MENINGITIS 12.0%	
CAUSES OTHER THAN DISEASE 7.1%	
TUBERCULOSIS 1.7%	
MEASLES 1.1%	
SCARLET FEVER .75%	
DIPHTHERIA .46%	
TYPHOID AND PARATYPHOID FEVER 1.4%	

6 WINTER MOS. SEPT. 29, 1917 TO MAR. 29, 1918.

Chart V.



Pneumonia has caused by far the greatest number of deaths in the army, and has been responsible for about eighty (80) per cent of total deaths caused by all the respiratory diseases. Comparative figures for army and civil life (for the age period 20 to 29 years) for deaths due to respiratory diseases are given in Table 7.

This table indicates that each respiratory disease, except tuberculosis, has caused an excessive fatality in army life. Assuming the conditions in the registration area for 1915 to be fairly representative of other years, we may express the relative fatality between civilian and army life during the six winter months as follows:

Pneumonia is	12	times	greater	in	the	Army.
Meningitis is	45	"	"	"	"	"
Measles is	19	"	"	"	"	"
Scarlet fever is	6	"	"	"	"	"
Diphtheria is	2	"	"	"	"	"
Tuberculosis is	13	"	"	"	Civil	Life.

It is evident that the low tuberculosis death rate in the army is due to the elimination of those with active forms of this disease. Furthermore, it is probable that most of the deaths from tuberculosis in the army have been due to the activation of inactive foci by acute respiratory diseases.

It is known that the winter covered by this report in the region east of the Rocky Mountains has been one of unusual severity and we might think that this has been partially responsible for the excessive rates in the army camps. A perusal of the figures, however, does not justify this speculation. For instance, in the following table there are given the death rates for pneumonia and meningitis during the past winter from those States from which we have been able to secure data.

TABLE 8

ANNUAL DEATH RATES PER 100,000 DURING SIX WINTER MONTHS  
(1915, January, February, March, October, November, December)

ALL AGES

(Instances in Black Faced Type Where Disease During the Past Winter Exceeded That in 1915)

PLACE	PNEUMONIA		MENINGITIS	
	1915	1917-18	1915	1917-18
Vermont	133	102*	6.1	.0*
Massachusetts	199	104*	7.9	4.2*
Rhode Island	202	183	6.6	6.3
Connecticut	190	220	10.3	3.8
New York City	227	265	4.7	4.3
Indiana	161	148	7.4	11.0
Michigan	134	152	9.7	9.6
Wisconsin	142	120	6.8	11.4
Minnesota	122	86	6.0	1.7
Kansas	133	143*	4.1	17.3*
Colorado	218	185	9.2	12.4
Montana	171	199	5.8	20.0
Virginia	161	158*	11.0	15.7*
Kentucky	141	169	13.5	22.0

\*NOTE: Vermont—Oct., Nov., Dec. only. Virginia—Oct., Nov., Dec., Jan. only. Kansas and Massachusetts—Oct., Nov., Dec., Jan., Feb. only.

Contrasted with the figures for the past winter are the rates for 1915. It will be seen that while the rates have been excessive in some States it is by no means common to all States. Thus the pneumonia rates during the past winter

have been higher than they were three years ago in Connecticut, New York City, Michigan, Kansas, Montana and Kentucky. Pneumonia rates have been lower this year than 1915 in Vermont, Massachusetts, Rhode Island, Indiana, Wisconsin, Minnesota, Colorado and Virginia. Of the fourteen places mentioned seven have had higher meningitis rates in the past winter, whereas the other seven have had lower. It is not evident therefore that the severity of the past winter has produced a bad effect on the pneumonia and meningitis incidence.

#### SICKNESS IN THE ARMY

While both pneumonia and meningitis have exacted the greatest toll in life, they have not been as important a factor in incapacitating troops as some other causes. Measles is a large factor in this connection. The annual mor-

### CAUSES OF ADMISSION TO HOSPITAL AND QUARTERS

29 NATIONAL GUARD AND NATIONAL ARMY CAMPS

6 MONTHS PERIOD. SEPT.29,1917 TO MAR.29,1918

OTHER DISEASES 75.5%	
MEASLES 7.3%	
VENEREAL DISEASE 5.8%	
CAUSES OTHER THAN DISEASE 4.8%	
PNEUMONIA 1.8%	
SCARLET FEVER .42%	
MENINGITIS 2.1%	
MALARIA .18%	
TYPHOID FEVER 0.19%	

Chart VI.

bidity rate for measles for all troops in this country during the six months' period was 105. per thousand. The rate for pneumonia was 24.2, that for meningitis 4.1. Pneumonia and meningitis are the more fatal diseases and therefore, although fewer cases occur, the number of deaths is great. The fatality rate, or case mortality rate, for all troops during the six months' period has been 23 per cent for pneumonia, 27 per cent for meningitis and 0.1 per cent for measles.

Most numerous as causes of sickness are those less fatal ailments such as colds, influenza, bronchitis, and mumps. Of the total cases admitted to hospital or quarters, relatively few are caused by the more serious ailments. Taking the National Guard and the National Army camps by themselves, we find that 95.2 per cent of admissions for sickness are due to disease of one form or

another. Of the more serious infectious diseases measles stands foremost, followed by venereal diseases. The relative incidence of the different diseases is shown in Table 9.

TABLE 9  
NATIONAL GUARD AND NATIONAL ARMY CAMPS  
SIX MONTHS' PERIOD, SEPTEMBER 29, 1917, TO MARCH 29, 1918  
CAUSE OF ADMISSION TO HOSPITAL OR QUARTERS

Causes other than disease <sup>1</sup>		4.8%
Measles	7.3	
Venereal disease	5.8	
Pneumonia	1.8	
Scarlet fever	.42	
Meningitis	.21	
Malaria	.18	
Typhoid and paratyphoid	.019	
Diseases other than the above <sup>2</sup>	79.5	
Total diseases		95.2%

\* (1) Under this heading are included injuries, sunstroke, sprained ankles, gunshot wounds, etc.

(2) This large group includes the minor and the rare diseases such as colds, la grippe, mumps, whooping cough, bronchitis, influenza, pharyngitis, laryngitis, tonsillitis, chickenpox, smallpox, anthrax, tetanus, etc.

The relative incidence of these various diseases in the different army groups is shown in the following table, where the morbidity from each cause has been expressed as an annual rate per 1000.

TABLE 10  
ANNUAL MORBIDITY RATE PER 1000  
SIX MONTHS' PERIOD, SEPTEMBER 29, 1917, TO MARCH 29, 1918

	MEASLES	VENEREAL DISEASE	PNEUMONIA	SCARLET FEVER	MENINGITIS	MALARIA	TYPHOID AND PARATYPHOID
Regular Army	57	77.2	17.6	9.9	1.9	1.3	.14
National Guard	132	75.4	31.8	3.0	2.8	3.6	.35
National Army	100	103	25.2	9.4	3.6	2.1	.24

NOTE: Data from 13 National Guard and 16 National Army camps.

The National Army with its new recruits fresh from civilian life shows the highest rate in venereal diseases and meningitis. National Guard troops, however, show the highest rates for measles, pneumonia, malaria and typhoid. Regular Army troops, representing those longest in the service and in all probability an older average age, show the lowest rates in everything but scarlet fever and venereal disease, the latter being slightly above the rate for the Guard.

#### EPIDEMIC DISEASE IN NATIONAL GUARD AND NATIONAL ARMY CAMPS

Measles, pneumonia, meningitis and scarlet fever have existed in epidemic form in a number of the camps. Measles has been by far the most prevalent of the four. Scarlet fever has been of minor consequence. Typhoid fever has been a negligible factor as a cause of incapacitation. Mumps, bronchitis and influenza have likewise been widespread and although not fatal in themselves, have frequently been forerunners of pneumonia.

#### PNEUMONIA

As pointed out elsewhere in this paper, pneumonia has been of more serious import than any other single disease. It has occurred in epidemic form in

many camps, particularly in those occupied by southern troops. In other camps, especially those occupied by the troops from the middle western and northern States, pneumonia has been sporadic rather than epidemic. Among the troops from the northeastern section of the country pneumonia has been less prevalent.

In Table 11 are presented the mortality and morbidity rates for pneumonia in the various National Guard and National Army camps.

TABLE 11  
SICKNESS AND DEATHS FROM PNEUMONIA IN ARMY CAMPS, SIX MONTHS' PERIOD,  
SEPTEMBER 29, 1917, TO MARCH 29, 1918

CAMP	ARMY	ANNUAL RATE PER 1000	
		MORBIDITY RATE	DEATH RATE
Bowie	N. G.	96.	20.0
Wheeler	N. A.	95.	23.6
Travis	N. A.	78.	10.6
Pike	N. A.	63.	24.9
Cody	N. G.	52.	9.7
Beauregard	N. G.	42.	15.0
Taylor	N. A.	37.	5.4
Sevier	N. G.	36.	11.5
Jackson	N. A.	35.	10.7
Doniphan	N. G.	33.	9.0
Dodge	N. A.	29.	5.3
Funston	N. A.	24.	10.5
Kearny	N. G.	24.	4.4
Lee	N. A.	24.	5.5
Shelby	N. G.	21.	4.7
Meade	N. A.	18.	2.6
Logan	N. G.	16.	1.0
Gordon	N. A.	15.	5.3
Sherman	N. A.	15.	2.5
Upton	N. A.	15.	3.6
Grant	N. A.	14.	1.5
Lewis	N. A.	11.	1.5
Devens	N. A.	9.8	2.0
McClellan	N. G.	9.6	1.1
Sheridan	N. G.	9.3	1.7
Wadsworth	N. G.	9.1	1.1
Dix	N. A.	8.0	1.5
Custer	N. A.	7.0	1.5
Hancock	N. G.	6.7	1.1

NOTE: Chart VII shows the morbidity from pneumonia by camps.

The highest pneumonia morbidity rates are at Bowie and Wheeler. These camps along with Pike likewise show by far the highest death rates from the cause. The excessive prevalence of this disease is appreciated on comparing the death rate at Pike with that for Logan, the lowest in the table. The rate at Pike is twenty-five times that at Logan.

As compared with civil life pneumonia has been unduly prevalent in the camps during the past winter. The average pneumonia rate for all camps is high, because of excessive rates at certain of the southern troop camps. The lowest camp rates are not higher than the rates for civilian life. The rate for the New York National Guard troops at Wadsworth, for instance, is 1.1, while the rate for the age group 20 to 29 years in New York City for the six winter months was 1.0. The rate for National Guard troops from Pennsylvania Camp Hancock is 1.1, as compared with a rate of 1.8 for the age group 20 to 29 years for the city of Pittsburg from October 1, 1917, to April 1, 1918. Near



## PNEUMONIA IN ARMY CAMPS

NATIONAL GUARDNATIONAL ARMY

WHEELER BOWIE

TRAVIS

PIKE

CODY

BEAUREGARD

SEVIER  
DONIPHAN

TAYLOR JACKSON

DODGE

KEARNY  
SHELBY

FUNSTON, LEE

LOGAN

MEADE  
GORDON, SHERMAN, UPTON GRANTSHERIDAN McCLELLAN  
WADSWORTH HANCOCKLEWIS DEVENS  
DIX CUSTER

ANNUAL MORBIDITY RATE PER 1000  
SEPT. 29, 1917 TO MAR. 29, 1918.

Chart VII.

twice as many deaths occurred in Pittsburg as in Pennsylvania men in Camp Hancock.

In New York City during the past winter the pneumonia rate for all ages is 2.65, for age group 20 to 29 it is 1.00. The proportion for the specific age group is thus 38 per cent. In Table 12 there are included pneumonia death rates for the past winter for a number of States. By applying the factor obtained in New York City to the death rates at all ages in other States we may secure a rough approximation of the death rates in age group 20 to 29 years.

TABLE 12  
PNEUMONIA DEATH RATES IN CIVILIAN LIFE  
ANNUAL RATE PER 1000  
PERIOD, WINTER OF 1917-1918

	MONTHS	RATE (ALL AGES)	RATE (AGE 20-29 YEARS)
Vermont	Oct., Nov., Dec.	1.00	.38
Delaware	Oct. to Mar., incl.	7.50*	2.8
Virginia	Oct., Nov., Dec., Jan.	1.58	.60
Connecticut	Oct. to Mar., incl.	2.20	.84
Massachusetts	Oct. to Feb., incl.	1.04	.40
Colorado	Oct. to Mar., incl.	1.85	.70
Michigan	Oct. to Mar., incl.	1.52	.58
St. Louis, Mo.	Oct. to Mar., incl.	2.80	1.1

\*This is the figure furnished us by the State Health authorities.

With the data secured in this manner we may strike the comparisons made in Table 12.

It is evident from Table 13 that Army rates are above those for civilian life. There is an exception in the case of Camp Dix whose rate is below that

TABLE 13  
COMPARATIVE MORTALITY RATES FROM PNEUMONIA IN ARMY AND CIVIL LIFE  
PERIOD, OCTOBER, 1917, TO APRIL, 1918  
(FIGURES IN ANNUAL RATES PER 1000)

CIVIL LOCALITY	PNEUMONIA RATE (ESTIMATED RATE AGE GROUPS 20 TO 29 YEARS)	PNEUMONIA RATE	ARMY CAMP	SOURCE OF TROOPS
Vermont	.38	2.0	Devens	New England States
Connecticut	.84			
Massachusetts	.40			
Delaware	2.8	1.5	Dix	Delaware, New York, New Jersey
Virginia	.60	5.5	Lee	Virginia, West Virginia, Pennsylvania
Colorado	.70	10.5	Funston	Colorado, Arizona, Kansas, Missouri, Nebraska, New Mexico, South Dakota
Michigan	.58	1.5	Custer	Michigan, Wisconsin
St. Louis, Mo.	1.1	9.0	Doniphan	Missouri, Kansas

of Delaware. Dix is occupied by New Jersey and New York troops as well as those of Delaware and the Dix rate is not therefore a fair representation for Delaware soldiers. In general, the camp rate for New England is about four times that of civilian life. Camp Lee is about nine times that of Virginia.

Funston is fifteen times that of Colorado. Custer is nearly three times that of Michigan. Doniphan is eight times that of St. Louis. We may conclude that in general camp life is more conducive to pneumonia than civilian life.

Pneumonia prevailed most extensively during November and December. The weekly pneumonia incidence for the army as a whole and for each of the Guard and National Army camps is pictured in Charts XX to XXXV accompanying this report. The rates which we have used in discussing this question are averages for a period of six months. The epidemic nature of the disease in some camps is suggested by the rates during the weeks when the disease was most prevalent. This is shown in Table 14.

TABLE 14  
PNEUMONIA MORBIDITY IN CERTAIN CAMPS  
FIGURES IN TERMS OF ANNUAL RATE PER 1000  
PNEUMONIA RATE

CAMP	AVERAGE SIX MONTHS	HIGHEST WEEK	WEEK ENDING
Bowie	96	468	Dec. 7
Wheeler	95	340	Nov. 23
Sevier	36	187	Nov. 23
Pike	63	172	Nov. 9

Camp Travis differs from the other camps in having a continuously high pneumonia rate and no exceptionally high peaks. Thus the average rate for the six months was 78, whereas the highest week was 169.

#### DIFFERENCES IN THE VIRULENCE OF THE INFECTING ORGANISMS CAUSING PNEUMONIA

The figures presented in this paper must forcefully impress one with the following facts:

1. Morbidity and mortality in the camps during the period covered by this report have been largely due to the acute respiratory diseases, especially pneumonia and meningitis, the former being the more potent factor in causing the high death rate.

2. The different camps have shown wide variations in morbidity and mortality from pneumonia.

With these facts in mind, one naturally asks, has the pneumonia in the different camps been the same disease or does this diagnostic term include two or more diseases, differing etiologically and possibly in other respects. May the wide differences especially in the mortality rates be explained by the predominance of one pneumonia in a given camp, while quite another disease under the same name has prevailed at another camp?

Clinically, pneumonia has been reported as lobar and broncho. The chiefs of the medical service in the Base Hospitals are men selected from the best clinicians in the country, and they recognize that a positive, sharp and uniform clinical differentiation between the forms of pneumonia is not at present easy. Accepting the diagnosis as reported, which form of pneumonia has been more prevalent and which more fatal? First, what evidence have we concerning the relative prevalence of these two forms of pneumonia?

TABLE 15

CAMP	PERIOD	TOTAL CASES	PER CENT OF LOBAR
Taylor	Oct.-Dec.	186	62
Pike	Oct.-June	528	67
Cody	Oct.-Mar.	537	93
Wheeler	Oct.-Apr.	775	86
Travis	Dec.	124	77
Travis	Apr.	42	64
Shelby	Mar.-Apr.	61	44

It is seen in Table 15 that at all these camps during the months mentioned with the exception of Shelby, lobar pneumonia predominated. It is reported from some camps that the dominant form of pneumonia changes with the arrival of new troops. Most of the camps, not all, report that bronchopneumonia has been the more fatal. The case mortality at Pike is reported at 57 per cent in broncho and 36 in lobar. At Cody the differences showed slightly higher mortality in the lobar form. It is generally agreed that bronchopneumonia is more frequently a secondary infection, especially after measles.

At Sevier there has been no recognized streptococcus pneumonia. The pneumococcus has been the causative agent in both lobar and bronchopneumonia and the prevailing organism has been Type IV.

In this camp a hookworm survey showed 18.6 per cent of the healthy soldiers infected with these parasites. Of total admissions to hospital 20 per cent were infected. Among the bronchopneumonia cases 50 per cent of those who died had hookworm, while among those who did not have this infection only 17 per cent died.

The Base Hospital laboratories have been well equipped and manned, and much valuable work has been done in the identification of the bacteria and the differentiation of the types of pneumonia. We are leaving to others a more complete digest of this work. In most of the laboratories Type IV has been reported as the predominating organism.

A more important matter is the prevalence of a pneumonia in which the streptococcus hemolyticus appears as the causative agent. This coccus is reported as causing both lobar and bronchopneumonia and the pneumonia caused by it appears to be more frequently complicated with empyema, and more fatal than that due to the pneumococcus.

The epidemiologist at Camp Upton reports as follows:

Case No. 1. The first case of streptococcus hemolyticus pneumonia to be admitted to the Base Hospital was a white man, assigned to the Depot Brigade, and sent to the hospital immediately upon his arrival on December 25, 1917, from Fort Slocum. He gave a history of having been seized with a severe chill and a sharp pain in the right lower chest, before leaving Fort Slocum. The streptococcus hemolyticus was identified in the laboratory two days after admission, and was later found in the empyemic fluid. This man died on January 17, and no other information concerning him is available, except that he was twenty-five years old and had been in the service less than one month. Following this initial case, there were 14 admitted to the hospital previous to March 1. Nine, or 64 per cent of these are colored, while during the same period the average strength of colored troops in camp was 3,380 or only about 10 per cent of the average strength of the camp. The troops stationed at Camp Upton during the winter were for the most part drafted men from the New York metropolitan area. A conservative estimate places the number of urban troops at 90 per cent of the command. In contrast to this, 56.5 per cent of the 48 cases of pneumonia due to the streptococcus hemolyticus admitted up to April were rural men.

Case No. 2 was a rural colored man from Texas, who arrived early in December from Camp Pike.



Case No. 3 was a rural colored man from Virginia, who had just arrived from Camp Lee.

Case No. 4 was an urban white man from Maine, who had been assigned to the Medical Department and who was on duty in the contagious section of the Base Hospital.

Case No. 5 was a colored Texas farmer, recently arrived from Camp Pike.

Case No. 6 was a Connecticut man, who arrived together with case No. 1 from Fort Slocum on December 25.

Case No. 7 was a colored Texas man, sent here from Camp Pike.

Case No. 8 which did not develop until January 28, was a white man from Cornwall, N. Y., on duty at the Officers' Training School.

These eight cases are all that developed prior to the first of February. It is seen from this that two of the first eight cases were imported from Fort Slocum, and three from Camp Pike, the latter camp having experienced much pneumonia during November and the first part of December. Camp Pike reported 316 pneumonia cases during this period. The early pneumonia cases at Upton among colored troops were all in the 367th Infantry, which drew its men for the most part from Camps Pike and Lee and New York City. The urban men from New York City remained practically uninfected. Having been introduced into Camp Pike, the virulent streptococcus infection spread gradually through the association of troops in camp until it assumed rather large proportions in March, with a total of 33 cases for that month. The mortality rate up to April 1st was 48 per cent.

We are convinced that the differences in morbidity and mortality from pneumonia in the camps are not explained on the ground of variations in kind or virulence of the infecting organisms.

#### DIFFERENCES IN SUSCEPTIBILITY TO PNEUMONIA OF THE MEN IN DIFFERENT CAMPS AND THE EXPERIENCES OF THE CIVIL WAR IN THIS CONNECTION

Our figures show most convincingly:

1. That men from rural communities are more susceptible to pneumonia than are those from urban life.
2. That on the whole the southern soldier is much more susceptible to this disease than his northern comrade.

The fact that pneumonia has been much more prevalent and much more fatal among southern than among northern troops has led us to look up the history of the acute respiratory diseases during the Civil War. Superficial examination of the "Medical and Surgical History of the War of the Rebellion"\* from which the quotations which follow are taken, shows that the acute respiratory diseases during the Civil War followed along the same lines as they have exhibited in our camps during the period covered by this report. We herewith present a brief résumé of the acute respiratory diseases as they appeared in the Civil War. During that war, there were reported in the Union Army 67,763 cases of measles with 4,246 deaths, giving a mortality rate of 6.27 per cent. It is more than probable that only a small part of this mortality was directly due to measles, but to certain sequæ, notably pneumonia. The average annual morbidity rate of measles per thousand of strength was 30.41, the maximum 7.57 during the first year, and the minimum 1.98 during the last year. Although there are no exact reports, it appears that measles prevailed in the Confederate Army and was much more highly fatal than in the Union Army. Prof. Paul F. Eve says concerning measles:

\*Part Third McNeal Volume.

"In the Confederate Army measles prevailed extensively in the new regiments, especially in those from the country, and greatly impeded their organization. It so diminished the effectiveness of the troops and proved so fatal in camps that companies, battalions and whole regiments had to be disbanded for a time and the men sent home." (p. 649.)

"As the new men came within the influence of the contagious foci, the disease spread, giving a sudden elevation to the line of prevalence, which therefore fell until fresh accessions occasioned a corresponding rise in level. The highest rates occurred in the early months when the commands were small and unprotected by previous attacks. In subsequent periods, the increased prevalence of rates, if calculated on the strength of the new regiments only, would probably have been equally high, but calculated, as they have been, on the mean strength, part of which had lost its susceptibility to the disease, they are necessarily lower than those of the earlier epidemic periods." (p. 649.)

"Recruits from the city are more likely to have passed through the disease in childhood than those from rural districts. City regiments are, therefore, to be preferred in this connection." (p. 659.)

The advisability of intentionally subjecting men to measles under proper sanitary conditions was considered:

"Inoculation for smallpox was practiced before the discovery of vaccination. However, the efforts of sanitary officers have been so successful in controlling the spread of communicable diseases that few medical men would counsel the intentional propagation of measles among large bodies of newly organized and susceptible troops." (p. 659.)

"Isolation proved ineffective in restricting the disease during the war, but there is no record of its having been systematically carried out." (p. 659.)

"Scarlet fever was rarely seen during the Civil War. Among the white troops 578 cases were reported, 70 of which or 12.1 per cent were fatal. Among colored troops the cases numbered 118 with but 2 deaths, equivalent to a mortality rate of only 1.7 per cent. This was, therefore, one of the exceptional diseases less fatal to the negroes than to the white men." (p. 662.)

"Mumps occurred to a notable extent immediately in the first year of the war, when 40 cases were reported among every one thousand men; the rate of prevalence fell to 23 in the second and third year, to 14 in the fourth year and to less than three in the fifth year." (p. 675.)

"The average annual morbidity from pneumonia among white troops was 27.8 and the death rate 6.21. Among colored troops the annual morbidity was 88 and the mortality 27.29." (p. 719.)

"Pneumonia was much more prevalent among the Confederate than among Federal troops. Among the former this disease annually affected 103 men of every one thousand, while the corresponding rate for Federal white troops was 34, and the cases reported as acute bronchitis and catarrhs numbered 415 yearly per thousand among Confederate troops as against 192 in the Union ranks." (p. 719.)

Among the Confederate troops operating in South Carolina, Georgia and Florida during the 19 months, January, 1862, to July, 1863, inclusive, there were 2,220 cases of pneumonia of which 127 terminated fatally in the field and 370 in the hospital, making a total of 497 deaths, equivalent to 22.4 per cent of the whole number of cases. (p. 720.)

A table shows a Confederate death rate from pneumonia of 20.6 per thousand strength as compared with the Union rate of 7.8 per thousand. The average annual rate of deaths from pneumonia among southern prisoners in northern camps was 59.9 per thousand, while the annual rate from the same cause at Andersonville was 27.4.

Acute bronchitis was reported as responsible for 168,715 cases of sickness in the Federal Army, and of these 650 terminated fatally. This applies only to white troops. Among the colored troops, the average annual rate for bronchitis was 123.5. (p. 726.)

Additional evidence of the greater susceptibility of the southern soldier to the acute respiratory diseases is supplied by the following figures (see Table 15-A), furnished by the "Sick and Wounded" Division of the Surgeon General's Office. These figures cover the calendar year of 1917, and they give the nativ

TABLE 15-A

LOBAR PNEUMONIA, BRONCHOPNEUMONIA AND MEASLES,  
AMONG U. S. TROOPS, ACCORDING TO NATIVITY  
ENLISTED MEN, YEAR 1917

State of Birth	ANNUAL ADMISSION RATE PER 1000*		
	LOBAR PNEUMONIA	PRIMARY BRONCHO- PNEUMONIA	MEASLES (UNCOMPLICATED)
Alabama	.233	.029	1.35
Alaska			.08
Arizona	.049		.22
Arkansas	.128	.065	1.36
California	.045	.013	.34
Colorado	.043	.015	.26
Connecticut	.028	.006	.08
Delaware	.020	.015	.13
District of Columbia			
Florida	.381	.042	1.45
Georgia	.153	.029	.88
Idaho	.043		.15
Illinois	.054	.008	.27
Indiana	.060	.017	.57
Iowa	.088	.010	.43
Kansas	.104	.013	.75
Kentucky	.111	.016	.82
Louisiana	.122	.056	1.14
Maine	.027	.008	.31
Maryland	.035	.009	.11
Massachusetts	.027	.004	.09
Michigan	.038	.007	.23
Minnesota	.043	.006	.21
Mississippi	.131	.038	1.26
Missouri	.101	.015	.61
Montana	.009	.001	.19
Nebraska	.128	.011	.49
Nevada	.049		.11
New Hampshire	.035	.012	.16
New Jersey	.022	.008	.06
New Mexico	.046	.018	.23
New York	.025	.006	.07
North Carolina	.056	.015	.79
North Dakota	.033		.22
Ohio	.042	.009	.18
Oklahoma			
Oregon	.042	.009	.31
Pennsylvania	.030	.006	.08
Rhode Island	.018	.007	.04
South Carolina	.079	.018	.66
South Dakota	.053	.005	.32
Tennessee	.099	.027	1.21
Texas	.231	.054	1.58
Utah	.032	.005	.21
Vermont	.005	.002	.40
Virginia	.039	.011	.36
Washington	.059	.005	.11
West Virginia	.030	.009	.28
Wisconsin	.050	.009	.44
Wyoming	.041		.25
Totals	.067	.014	.43

\*Number of admissions for men of any one state divided by the population of that state, times 1000.

ate of all soldiers who had measles or pneumonia during that year, quite independently of the organizations to which they belonged or the locations where they became ill. These figures have great relative value and show most convincingly the greater susceptibility of southern men to these diseases. It must

not be inferred that this higher susceptibility is inherited. At least such an assumption is not warranted, since it is more than probable that most of these men lived for the greater part of their lives in the states in which they were born. For the proper interpretation of these figures, we may say that during the year 1917, Alabama furnished out of every 100,000 of its population, 135 soldiers who contracted measles during that year.

It will be seen from the above that then, as now, measles, mumps and pneumonia were highly prevalent among the troops, both North and South, but that measles and pneumonia were much more prevalent and much more fatal among southern than among northern soldiers. Sickness and death in camp have been greatly lessened since Civil War days as Chart VIII will show.

If we can explain why the rural man is much more susceptible to pneumonia than his urban brother and why the southern soldier is much more susceptible to the same disease than his northern comrade, we will have a basis on which to work in our attempts to lessen the morbidity and mortality from pneumonia in our camps. We are aware that we are only on the threshold of this investigation and that more extensive and exact data may modify our views, possibly radically change them, but at the risk of falling into error, we are venturing certain suggestions.

In the first place we recognize that no sharp line can be drawn between rural and urban life. It is customary to classify those communities with a population of less than 2,500 as rural, but this is arbitrary and we must recognize the fact that our population is not fixed but is in a condition of constant flux. Because a soldier comes from a farm does not mean that he has not passed much of his life, even recently, in some crowded community, but the large masses of men with which we are dealing may justify a rough classification into rural and urban. Camp life is more crowded than that of most conditions of civilian life, even in the most densely congested areas of our largest cities. When we speak of "crowding" in camp, we are most likely to have in mind sleeping quarters and we demand in these not less than 50 square feet of floor space per man, but while proper space in sleeping quarters is a matter of importance men may be dangerously crowded even out of doors. The acute respiratory diseases are transmitted by the transference of organisms from the respiratory tract of one man to those of another. To put it bluntly, "by spitting into one another's face," and this occurs more frequently in the waking than in the sleeping hours. Crowding in camp life is a military necessity. It may be reduced to a minimum, and every effort should be made to do this. In an assembly hall in one of our camps where several thousand men are seated night after night, if every man sits upright and moves his head neither backward nor forward, the greatest distance between his nose and that of the man in front or behind is 26 inches, and to right or left, 16 inches. In such an assembly with one-half the men coughing, one can have some idea of the extent to which respiratory bacteria are being transmitted. This condition would not be materially altered by the removal of the walls and roof of the hall. On the other hand, the transfer of respiratory bacteria may be quite indirect. They may be carried in dust, and it is not surprising that the acute respiratory diseases have increased after dust storms as has been shown to be the case at Funston, Bow and at other camps.



# THE FRUITS OF PREVENTIVE MEDICINE

BY MAKING USE OF OUR INCREASING KNOWLEDGE  
OF MEDICINE AND SANITATION SINCE CIVIL WAR  
TIMES IT HAS BEEN POSSIBLE TO PREVENT  
HALF A MILLION CASES OF DISEASE  
AND SAVE THE LIVES OF  
TEN THOUSAND SOLDIERS

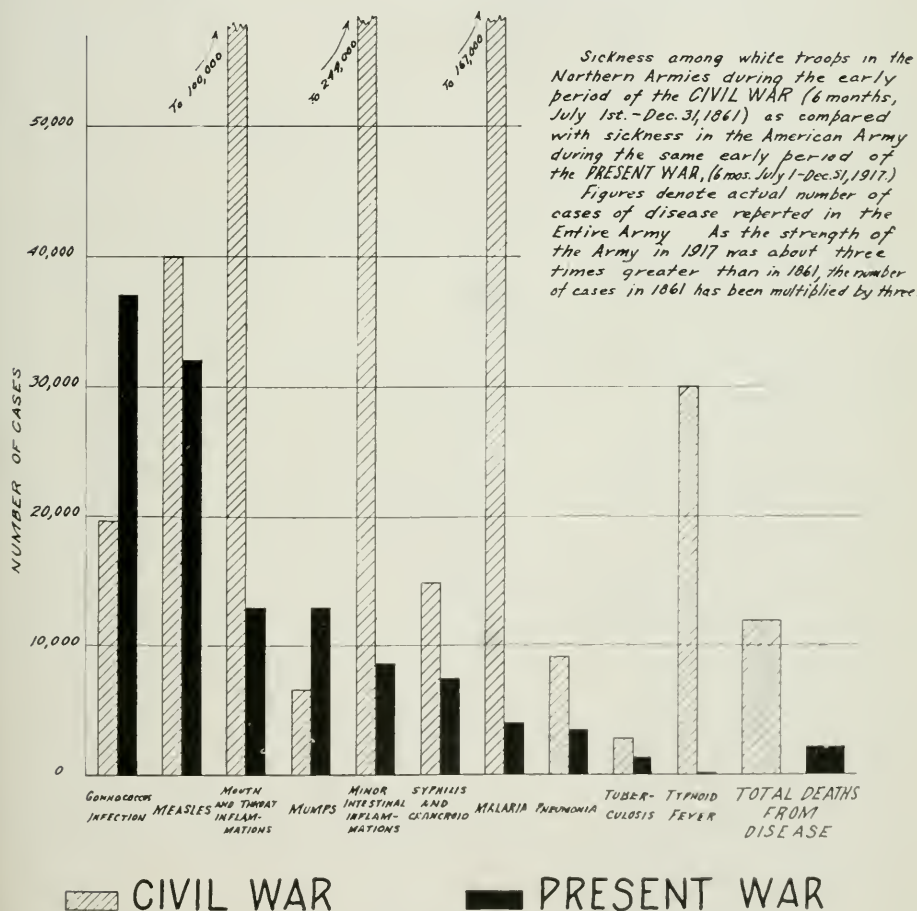


Chart VIII.

Pneumonia is an urban or a crowd disease in both civilian and military life. Our camps are the most crowded cities in our country, and consequently they furnish the highest rates for the respiratory diseases.

The man who has lived in a densely populated area is more resistant because he has been exposed to the same bacteria before, probably many times, and has acquired more or less immunity or an increased resistance. For a converse reason, the man who has lived in a sparsely settled community is the more susceptible because he has never before, or has not recently, harbored these bacteria. We call attention to the maps showing the location of the camps and areas from which their soldiers were drawn. (See Charts XVIII and XIX.) The soldiers less susceptible to pneumonia are from the most densely populated areas.

In the South, under ordinary conditions of civilian life, pneumonia is relatively rare, but when it does appear, is highly fatal, and is highly fatal because it is rare. The average southern lad has never come, or but seldom been brought, into contact with the organisms which cause pneumonia and when they are sprayed into his face, he falls a ready victim just as the American Indians and Fiji Islanders were decimated by measles and, as smallpox aided the Spaniards in their conquest of Mexico. Many illustrations along this line will occur to the student of epidemiology.

#### MEASURES FOR PROTECTION AGAINST PNEUMONIA AND OTHER RESPIRATORY DISEASE

In our efforts to lessen morbidity and mortality from the acute respiratory diseases, especially pneumonia, we must endeavor to develop the resistance of the soldier to the causative organisms. In the accomplishment of this purpose many procedures are possible. Up to the present time we have relied upon attempts to limit the number of infecting organisms finding access to the susceptible man by wearing masks, by placing the sick in cubicles, by the employment of disinfecting agents, etc. These methods have probably been of value—how much we can not say—but they are applicable only to the sick in hospitals, in detention camps or in quarters, and are not applicable to the great masses of the soldiers. The most scientific and the most promising procedure lies in vaccination. This has been tried already in some camps. At Upton, more than 12,000 out of a division of about 30,000 were vaccinated against pneumonia, and during the following two months that the practically unbroken division was under observation, the protective value of the vaccination seemed to be quite in evidence. In other camps, vaccination has been cautiously used, but up to the present time there have been established no standard methods, and it would be premature to make any definite claims. It is worthy of note that in South Africa vaccination against pneumonia has been reported with at least the promise of success. Enough has been done along this line to demonstrate that vaccination with pneumocci, properly done, results in no injury. In this procedure, the number and virulence of the organisms introduced are absolutely under control. It is the scientific method of increasing resistance. It is not probable that the increased resistance to pneumonia secured by any method continues indefinitely, since one attack of the disease does not protect for any great length of time, at least, against subsequent infection, but temporary advances in resisting power would be of great service.

We might learn something from the natural process by which increased resistance to the acute respiratory diseases is developed. This apparently is secured by gradual adaptation to the conditions of crowd life. Instead of taking our new soldiers from the comparative isolation of rural life and placing them immediately in crowded camps, they should be assembled in small groups at their homes, drilled and prepared for camp life more gradually. There should be a reserve army and for every man sent to a training camp, two should be added to the reserve army. These men should be permitted to continue their civilian functions, but should wear a distinctive uniform, be under strict discipline, have their vaccinations and drill in squads of gradually increasing size and thus slowly be introduced to the conditions of crowd life.

The difference between the "raw" and "seasoned" soldier so far as susceptibility to the infectious diseases is concerned is in our opinion explainable as here suggested. The former is without previous recent exposure to "crowd" bacteria and falls a ready victim. The latter, on account of frequent exposure to "crowd" bacteria shows a certain degree of immunity or at least increased resistance. The present method of mobilization is conducive to the spread of the acute respiratory diseases. For instance, select men in Florida are assembled at certain points in that state, and shipped to Camp Wheeler in Georgia in troop trains. It has appeared that as many as 6 cases of fully developed measles have been found on one of these trains on its arrival at Camp Wheeler. Those reaching the camp not actually ill are sent to the Depot Brigade or a Detention camp. On a certain day a colored contingent of drafted men from Alabama who had never been in a camp, reached Camp Custer in Michigan, and were placed in the Depot Brigade. During the following month, the epidemiologist at Custer reported "80 per cent of the cases of pneumonia arose in the Depot Brigade, which is the receiving place and also the distributing point for arriving recruits." Moreover, the type of the dominant pneumonia at Custer changed after the arrival of the Alabama troops. This experience has a parallel in many camps. It is worthy of note that of the 86 cases of pneumonia reported from Custer for the month following the arrival of the Alabama contingent, 69 came from the Depot Brigade, and of these 50 from the Alabama contingent.

The plan of having several hundred naked men closely crowded pass through the physical tests appears to be, at present, a military necessity, but that it affords ready means for the distribution of the acute respiratory diseases, is evident. It would be far better if all these examinations, vaccinations, etc., could be done in the reserve army which has been suggested. The transfer from the comparative individualism of civilian life to the intense communism of military life should be more gradual. The impossibility of bringing an effective army into existence "over night" is as evident to the sanitarian as it is to the military man.

To hope to reduce infection among our soldiers to the minimum without adequate attention to the health of the civilian population from which the soldier comes and with which he mingles more or less freely is without justification. If it be wise to protect the soldier by compulsory vaccination against smallpox and typhoid fever, why is it not right to protect the prospective soldier, the young man who is to be called next month or next year, against the same infections in the same way? All the infections which have appeared in the camps are dis-

tributed among the civilian population from which the soldiers in the camp have come. As an illustration, the figures in Table 16 show the present prevalence of infections in North Carolina. The figures indicate new cases reported every week

TABLE 16

INFECTIOUS DISEASES IN THE STATE OF NORTH CAROLINA FROM APRIL 6 TO JUNE 29, 1918

	APRIL				MAY				JUNE				
	6	13	20	27	4	11	18	25	1	8	15	22	29
Whooping cough	177	198	273	263	337	298	387	355	467	567	443	993	476
Measles (both kinds)	338	365	337	386	325	201	275	298	211	149	138	95	93
Diphtheria	18	9	19	14	10	16	12	16	8	8	9	14	10
Scarlet fever	11	6	4	10	2	8	7	8	7	2	11	15	18
Septic sore throat									5	6	5	1	5
Smallpox	31	13	18	32	36	52	14	15	18	11	13	17	17
Chickenpox								14	15	21	16	7	5
Infantile paralysis								3	1			1	1
Typhoid fever	10	8	6	6	7	12	11	12	17	29	48	76	98
Meningitis	5	2	3	6	1	3	2	5	1	3	3	1	1

Draft men from all parts of North Carolina are being sent to Jackson and Sevier and carry the infections with them. These arrivals consist of infected and susceptible material. They carry both the spark and fuel. If these men were organized and drilled in small squads at home and gradually inducted into military life, and only those found to be free from infection sent to the camps, the control of communicable disease among the soldiers would be greatly aided.

#### MEASLES

The morbidity from measles presents an entirely different picture from that of pneumonia. The highest morbidity rate from pneumonia was fourteen times that of the lowest. In the case of measles the highest rate was 62 times that of the lowest. Measles is thus seen to be much more epidemic in character. It exhibits an explosive characteristic in the suddenness with which it breaks forth and recedes. This is illustrated in the case of Camp Wheeler. The dates and morbidity rates were as follows:

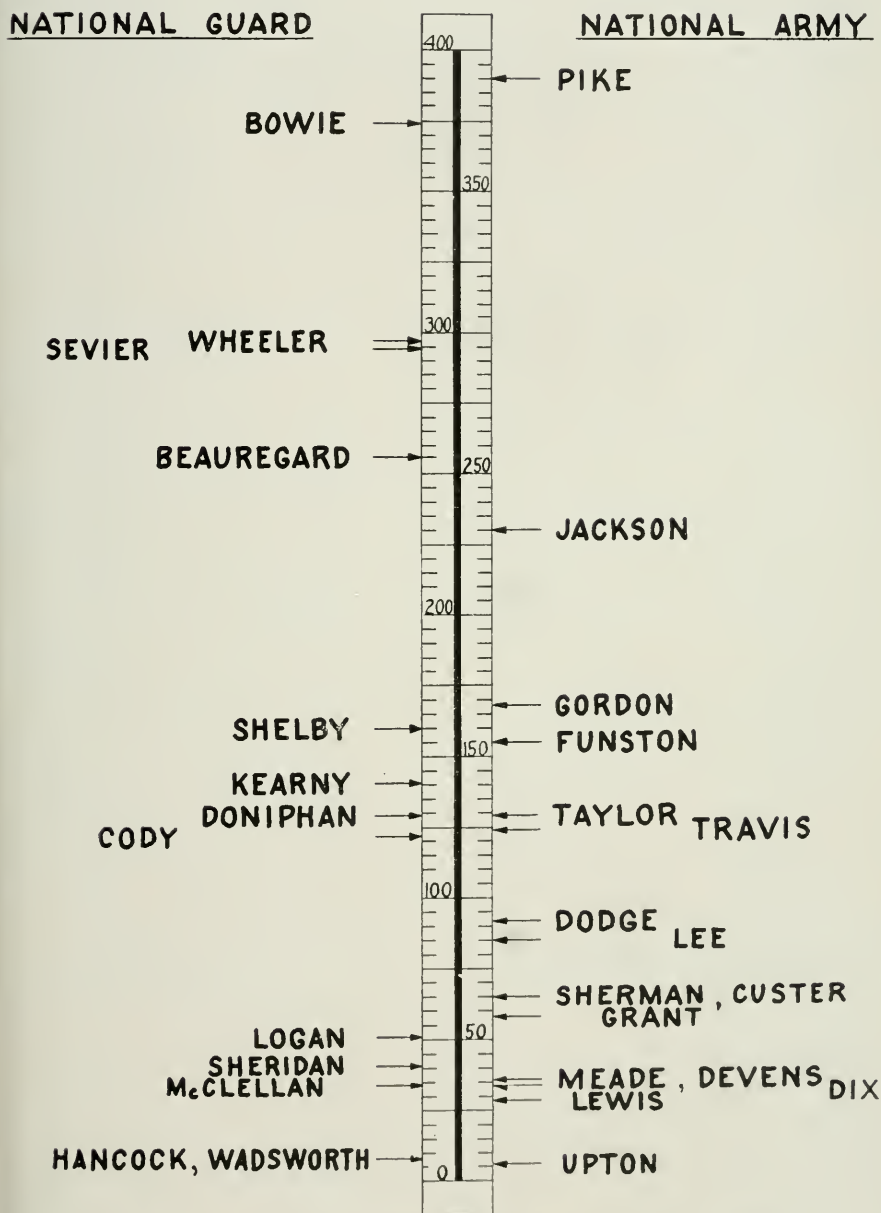
TABLE 17

FOR WEEK ENDING	ANNUAL MEASLES MORBIDITY RATE PER 1000
Oct. 19	83
Oct. 26	428
Nov. 2	615
Nov. 9	1760
Nov. 16	2200
Nov. 23	1120
Nov. 30	248
Dec. 7	240
Dec. 14	19

Measles was present to an excessive degree in about the same camps that experienced trouble from pneumonia. Southern troops suffered most severely from this sickness. The mortality from measles is of course a minor factor when compared to that from pneumonia. Measles, however, has been a serious disease because in addition to the time lost and the great numbers incapacitated,



## MEASLES IN ARMY CAMPS



ANNUAL MORBIDITY RATE PER 1000  
SEPT. 29, 1917 TO MAR. 29, 1918.

Chart IX.

it has been a forerunner of pneumonia in many instances. We are still in doubt concerning the relation between these diseases.

From statistics collected during the month of February at Camp Wheeler, it was found that 14 per cent of the pneumonia cases were preceded by measles. It is believed that this figure is much higher in some of the other camps. Including measles, mumps and colds of one kind or another, this group of diseases preceded pneumonia in 68 per cent of the pneumonia cases at Wheeler.

The average morbidity rates for measles in each camp, as well as the mortality rates, are given in Table 18.

TABLE 18

MORBIDITY AND MORTALITY FROM MEASLES IN ORDER OF PREVALENCE AT NATIONAL GUARD  
AND NATIONAL ARMY CAMPS

(Six Months' Period, September 29, 1917, to March 29, 1918)

FIGURES REPRESENT ANNUAL RATE PER 1000

CAMP	ARMY	MORBIDITY RATE	DEATH RATE
Pike	N. A.	390	.15
Bowie	N. G.	374	0
Wheeler	N. G.	297	0
Sevier	N. G.	294	.15
Beauregard	N. G.	256	.11
Jackson	N. A.	230	.33
Gordon	N. A.	168	.21
Shelby	N. G.	160	.16
Funston	N. A.	155	.07
Kearney	N. G.	141	0
Taylor	N. A.	129	0
Doniphan	N. G.	129	.08
Travis*	N. A.	124	0
Cody	N. G.	122	0
Dodge	N. A.	92	0
Lee	N. A.	85	.19
Sherman	N. A.	65	.38
Custer	N. A.	65	.49
Grant	N. A.	58	.08
Logan	N. G.	51	0
Sheridan	N. G.	41	0
Meade	N. A.	36	0
Devens	N. A.	36	0
McClellan	N. G.	34	0
Dix	N. A.	34	0
Lewis	N. A.	28	0
Hancock	N. G.	8	0
Wadsworth	N. G.	7.9	0
Upton	N. A.	6.3	0

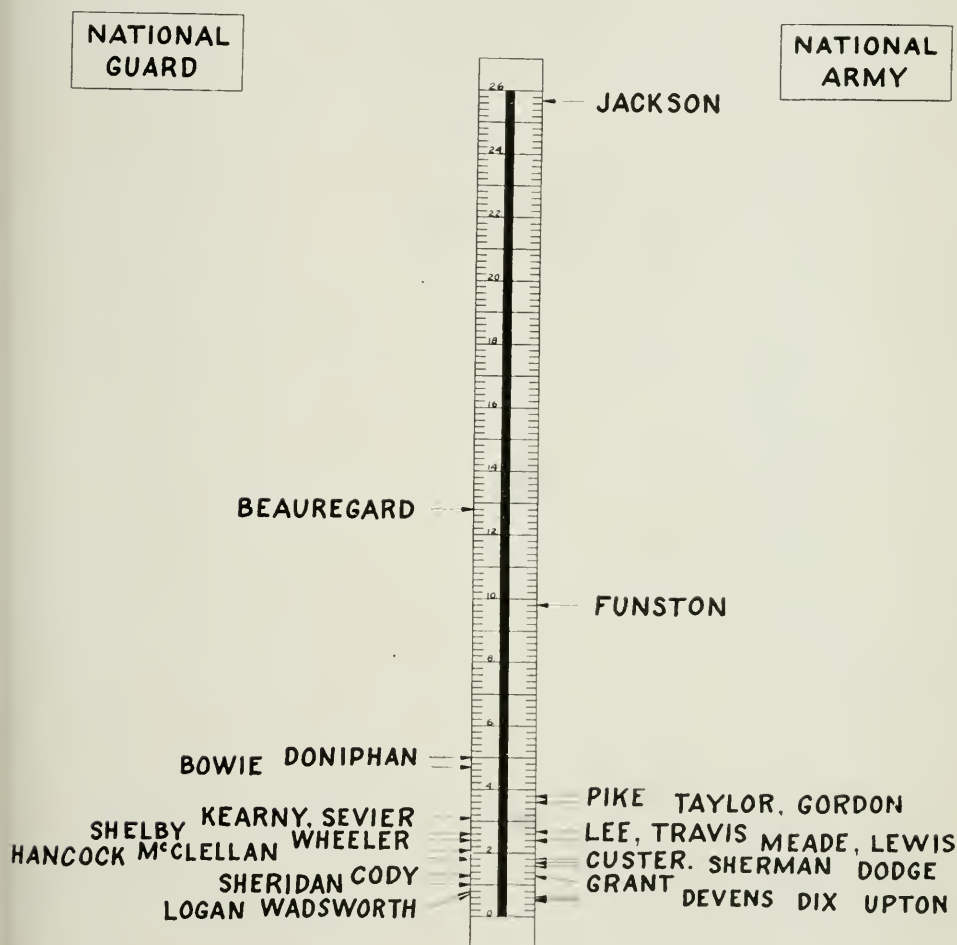
\*This rate is lower than it should be as many cases were not reported in the fall of 1917. See Chart IX for Measles morbidity in camps.

The highest mortality rate from measles was at Camp Custer. Even this rate is small, compared to that for pneumonia. It may be pointed out that many camps with high measles rates show few or no deaths from this cause. This is explained by the fact that the more serious cases were complicated with pneumonia and the death charged to this cause instead of measles. This is a more probable explanation than any difference in virulence of the infecting organism. It seems highly improbable that the case mortality rate at Camp Bowie should be zero while that at Camp Custer was about 0.8 per cent. With a case mortality rate of 0.8 per cent at Bowie we should have had a death rate of about 3.0 per 1000.

Comparative measles statistics for civilian life are obtainable only in an

indirect manner. The morbidity rates from this cause for the six winter months of the past year range from 1.0 per 1000 for the State of Vermont to 12.4 per 1000 for the State of Virginia. These rates are for people of all ages. Inasmuch as the mortality from measles for the age group 20 to 29 years is but a few per cent of that for all ages, it is probably that the morbidity rates for the States and cities rarely approach 1.0 per 1000.

## MENINGITIS IN ARMY CAMPS



**ANNUAL MORBIDITY RATE PER 1000**  
**SEPT. 29, 1917 TO MAR. 29, 1918**

Chart X.

As the morbidity rate for all National Guard and National Army camps was over 6.0, it is quite evident that measles has been appreciably greater in the army. The weekly incidence of measles has been pictured on the charts for pneumonia already mentioned.

## MENINGITIS

Meningitis in its various forms has appeared in every camp during the six winter months. The prevalence of this disease at Camp Jackson, however, has stood out above that of all other camps. The morbidity here has been just twice that of the camp having the next highest rate. Next to Jackson stand Beauregard and Funston. These rates are 25.7, 12.8 and 9.8, respectively. Next comes Doniphan with 5.0 and the other camps follow with rates at close intervals, the figures receding gradually from this point. (See Table 19.)

TABLE 19

MENINGITIS (ALL FORMS) IN NATIONAL GUARD AND NATIONAL ARMY CAMPS  
FIGURES REPRESENT ANNUAL RATE PER 1000

CAMP	ARMY	MORBIDITY RATE	DEATH RATE
Jackson	N. A.	25.7	7.5
Beauregard	N. G.	12.8	7.7
Funston	N. A.	9.8	3.6
Doniphan	N. G.	5.0	1.8
Bowie	N. G.	4.7	1.6
Pike	N. A.	3.8	2.4
Taylor	N. A.	3.6	1.3
Gordon	N. A.	3.6	.96
Sevier	N. G.	3.1	1.1
Kearny	N. G.	3.1	.77
Lee	N. A.	2.7	1.2
Travis	N. A.	2.7	2.5
Shelby	N. G.	2.6	.71
Wheeler	N. G.	2.4	2.1
Meade	N. A.	2.4	.66
Lewis	N. A.	2.4	.67
McClellan	N. G.	2.1	.41
Hancock	N. G.	1.8	.44
Custer	N. A.	1.8	.30
Sherman	N. A.	1.7	.32
Dodge	N. A.	1.6	1.1
Cody	N. G.	1.3	.50
Grant	N. A.	1.3	.31
Sheridan	N. G.	1.0	.18
Wadsworth	N. G.	.78	.20
Logan	N. G.	.73	.22
Devens	N. A.	.63	.28
Dix	N. A.	.57	.19
Upton	N. A.	.54	.34

(See Chart X for Meningitis Morbidity in camps.)

The three camps with highest morbidity rates likewise have the highest death rate. Beauregard, however, shows a death rate even greater than Jackson. Again, it is the southern troops who have suffered most severely from this disease. Those escaping it are the troops from the northeastern and northern sections of the country.

Meningitis, next to pneumonia, has been the most serious disease that the Medical Corps of the Army has had to meet. It is serious by reason of its high fatality and also because this of all diseases shows the greatest excess over the disease in civilian communities. It will be recalled from the early part of this report that meningitis was estimated to be 45 times as prevalent in the Army as in civilian life, whereas the figure for measles was 19 and for pneumonia 12. For this past winter we have the figures only for New York City and Pittsburgh, within the age group 20 to 29 years. The annual death rate for meningitis per



1000 was .038 for New York and .047 for Pittsburgh. The death rate for all ages in New York City was .043. The rate for age group 20 to 29 years is thus 88 per cent of that at all ages. Applying this factor to the rates in the various states we can obtain a rough approximation of the rates for the specific age group in these states. (See Table 20.)

TABLE 20  
MENINGITIS IN CIVIL COMMUNITIES  
FIGURES REPRESENT ANNUAL DEATH RATE PER 1000

PLACE	RATE FOR ALL AGES	RATE FOR AGE 20 TO 29 YEARS (COMPUTED BY USING NEW YORK CITY FACTOR OF 88%)
Vermont	.0	.0
Virginia	.16	.14
Delaware	.12	.11
Connecticut	.038	.034
Massachusetts	.042	.037
Colorado	.12	.11
Michigan	.096	.085
St. Louis, Mo.	.047	.042
New York City	.043	.038

Accepting the rates thus obtained for what they are worth, we may contrast these figures with those for various army camps. (See Table 21.)

TABLE 21  
MENINGITIS IN CIVIL LIFE (AGE 20 TO 29 YEARS) AND ARMY CAMPS  
ANNUAL DEATH RATES PER 1000  
Six Winter Months, 1917-18

PLACE	RATE	RATE	CAMP	SOURCE OF TROOPS
Vermont	.0	.024	Devens	New England States
Connecticut	.034			
Massachusetts	.037			
Virginia	.14	1.2	Lee	Virginia, West Virginia, Pennsylvania
Delaware	.11	.19	Dix	Delaware, New Jersey, New York
Colorado	.11	3.6	Funston	Colorado, Arizona, Kansas, Missouri, Nebraska, New Mexico, South Dakota
Michigan	.085	.30	Custer	Michigan, Wisconsin
New York City	.038	.27	Wadsworth	New York
		.34	Upton	New York
Pittsburgh	.047	.44	Hancock	Pennsylvania

It is evident from Table 21 that there is an excess of meningitis even in those camps which have been unusually free from pneumonia and measles. Camp Dix has next to the lowest death rate of all the camps, namely, .19 per 1000. People of age group 20 to 29 in the state of Delaware show a rate for the past six months of .11 per 1000. The camp rate is nearly twice as great as a comparable civil community in this instance. Camp Devens has more than ten times the rate of certain New England States. Camp Lee has nearly ten times the rate of Virginia. Camp Funston has more than thirty times the rate of Colorado. Custer has three and a half times the rate of Michigan. Camps Wadsworth and Upton are seven times greater than New York City. Camp Hancock has nearly ten times the rate of Pittsburgh. If the rate for Louisiana is at all like that for Virginia, namely .14 per 1000, we find that Camp Beauregard's rate of 7.7 would be in the ratio of 55 to 1.

It seems quite evident that meningitis has been most prevalent in those camps whose soldiers come from areas in which this disease has been endemic. For some years meningitis has been widely scattered in South Carolina and adjoining states and it appeared in the families of the workmen engaged in building the barracks at Camp Jackson before the troops began to assemble and during the period covered by this report it was reported in many places in South Carolina. Meningitis has been endemic in Missouri and Kansas for some years and this accounts for its presence at Funston. Here also it appeared in the workmen and their families and in villages near Funston. We have no data concerning meningitis in the civil communities from which the soldiers at Beauregard came. The behavior of this disease is a strong argument for a reserve army and for culturing throats before the draft men leave their homes.

In the last three months some thirty or more localities in South Carolina have reported meningitis to the State Board of Health. During this time men from all parts of the State are being sent to Camp Jackson. It must happen that many of them from the infected localities are meningitis carriers. These are less likely to develop the disease than some of those with whom they come in contact. If the drafted men, especially those from the infected localities, were collected in small groups at or near their homes and held until cultures were made and no carriers sent to camp, in our opinion this disease would be greatly reduced among our soldiers. As it is, one carrier transmits this infection to others and in a short time there are many carriers.

#### SCARLET FEVER

Scarlet fever manifested itself for the most part in Camps Pike, Lewis, Kearny, Sherman, Dodge and Grant. The incidence at Pike stands out by itself, however, as is shown in Table 22. The rate at Pike is nearly twice that of the second camp in the table. Scarlet fever presents a most interesting problem for the student of community diseases, inasmuch as the rate in the National and Regular Army is three times that in the National Guard (See Table 8), whereas pneumonia and measles on the other hand showed an appreciable excess in the Guard.

The National Army rate is elevated mainly by the camps mentioned above. It is significant that southern camps, with the single exception of Pike, have been free from this disease. The camps most affected have been those containing troops from the Middle and Far West, Lewis, Kearny, Dodge, Grant, Custer. Meade, Dix and McClellan are also among the first ten of the table. Another point brought out by the table is that of the nine camps having the highest morbidity rates, eight are National Army camps. But one camp, namely, Kearny, is a Guard camp.

Just why scarlet fever should be distributed in the above manner it is very difficult to say. The primary difference between Guard and Nationals is in their housing, the former being quartered in tents, the latter in barracks. The nature of the quarters would not seem to be a factor, or we should expect some of the eastern and southern National Army camps to show scarlet fever, which they do not.

It seems most probable that this peculiar distribution is attributable to the presence of the disease in those particular localities from which these troops are

TABLE 22  
SCARLET FEVER IN ARMY CAMPS  
ANNUAL RATES PER 1000

Six Months' Period, September 29, 1917, to March 29, 1918

CAMP	ARMY	MORBIDITY RATE	DEATH RATE
Pike	N. A.	43.8	.44
Lewis	N. A.	25.1	.12
Kearny	N. G.	19.3	.0
Sherman	N. A.	17.3	.19
Dodge	N. A.	15.8	.0
Grant	N. A.	12.3	.23
Meade	N. A.	7.7	.07
Custer	N. A.	6.5	.20
Dix	N. A.	6.4	.09
McClellan	N. G.	3.7	.0
Doniphan	N. G.	3.6	.0
Funston	N. A.	3.6	.0
Cody	N. G.	2.4	.08
Taylor	N. A.	2.4	.0
Sevier	N. G.	1.9	.15
Sheridan	N. G.	1.8	.0
Hancock	N. G.	1.5	.06
Devens	N. A.	1.5	.0
Upton	N. A.	1.5	.0
Logan	N. G.	1.4	.0
Jackson	N. A.	.9	.0
Wadsworth	N. G.	.8	.0
Lee	N. A.	.8	.0
Bowie	N. G.	.6	.08
Shelby	N. G.	.6	.0
Travis	N. A.	.4	.0
Gordon	N. A.	.1	.0
Beauregard	N. G.	.0	.0
Wheeler	N. G.	.0	.0

drawn. This would account for the disease in both Kearny and Lewis, which contain men from the same general locality. The limitation of the disease among the National Army troops and its relative exclusion from the Guard, may possibly be explained by the later assembling of the Nationals, who brought scarlet fever into the camp after it had developed in the civilian communities. Furthermore, it may be pointed out that there are no Guard camps in the North. Therefore, the Guardsmen on mingling with the civilians in their vicinity were not brought into contact with the disease as frequently as the men from the camps located in the North. This explanation rests on the assumption that scarlet fever prevailed more extensively in the neighborhood of the northern camps than elsewhere. This fact is borne out by Table 23.

These data are very suggestive. Scarlet fever is seen to be much more prevalent in the middle and far West than in the South or East. It was highest in Montana, a State that contributed men to Camp Lewis. It was high in Michigan, Minnesota and Wisconsin which contributed men to Camps Grant and Custer. It was high in Delaware which sent men to Camp Dix. Camp Sherman's high position in the table is probably due to scarlet fever in Ohio. We do not have death rates for Ohio but from the morbidity statistics available, Ohio is seen to have a rate almost as high as the States of Michigan and Wisconsin. Records from the territory contributing to Pike are not available so that nothing can be said as to the influence of civilian diseases on the scarlet fever incidence at Pike.

TABLE 23  
SCARLET FEVER IN CIVIL LIFE  
ANNUAL DEATH RATES PER 100,000  
Six Winter Months, 1917-1918

STATE	DEATH RATE	AVERAGE DEATH RATE FOR SECTION
Vermont	3.3	3.4
Massachusetts	3.3	
Rhode Island	5.7	
Connecticut	1.4	
New Jersey	2.5	4.5
Delaware	6.5	
Indiana	6.3	6.3
Michigan	7.3	
Wisconsin	7.2	
Minnesota	6.6	
Kansas	3.9	
Colorado	1.8	13.2
Montana	24.6	
Maryland	1.6	1.4
Virginia	1.0	
Kentucky	1.5	

DIFFERENCES IN THE BEHAVIOR OF MEASLES, PNEUMONIA, MENINGITIS, AND  
SCARLET FEVER

Certain differences in the characteristics of the diseases above mentioned are revealed in Chart XI, where there are plotted the morbidity rates for the National Guard and National Army Camps combined. The scale of the plot has been adjusted so as to bring the curves of each disease near together, otherwise there would be little opportunity for comparison, as the meningitis curve would scarcely appear at all if plotted to the scale for measles. These curves are designed to show the seasonal occurrence of the disease.

In the upper diagram there will be noted a surprising similarity between pneumonia and meningitis. Although pneumonia was about ten times as prevalent as meningitis, both diseases progressed at about the same rate and except for a period during December and January are parallel throughout the six months' period. In a sense their occurrence has been epidemic in nature as there is a distinct though blunt peak to the curves. The breadth of this peak extends from November to February with the greatest incidence during January and February.

The upper curves present a far different appearance from those of measles and scarlet fever. The measles curve is distinctly epidemic in character. It exhibits a sharp peak. The disease increased enormously during November and started to recede just as sharply. Then its rate of recession decreases and it flattens out very gradually reaching a level the first of March.

Scarlet fever acted differently from the other three diseases. Beginning in October it gradually ascended and did not reach its maximum height until fifteen weeks later. Aside from the fact of its gradual ascent, the scarlet fever curve resembles pneumonia and meningitis more closely than it does measles.

There are many factors which have governed the incidence of these various diseases. In these curves, measles stands out as a disease of distinctly epidemic character, a disease that is highly infectious and spreads rapidly. It is only



# SEASONAL OCCURRENCE OF COMMUNICABLE DISEASE IN NATIONAL GUARD AND NATIONAL ARMY CAMPS

6 WINTER MONTHS, SEPT. 29, 1917 TO MAR. 29, 1918.

NOTE: Disease incidence is expressed in terms of Annual Rate per 1000. Measles has been plotted to scale. Scarlet Fever rates have been multiplied by 25, Pneumonia rates by 5, and Meningitis rates by 50.

It will be noted that Pneumonia and Meningitis show similar characteristics, quite different from the curves below.

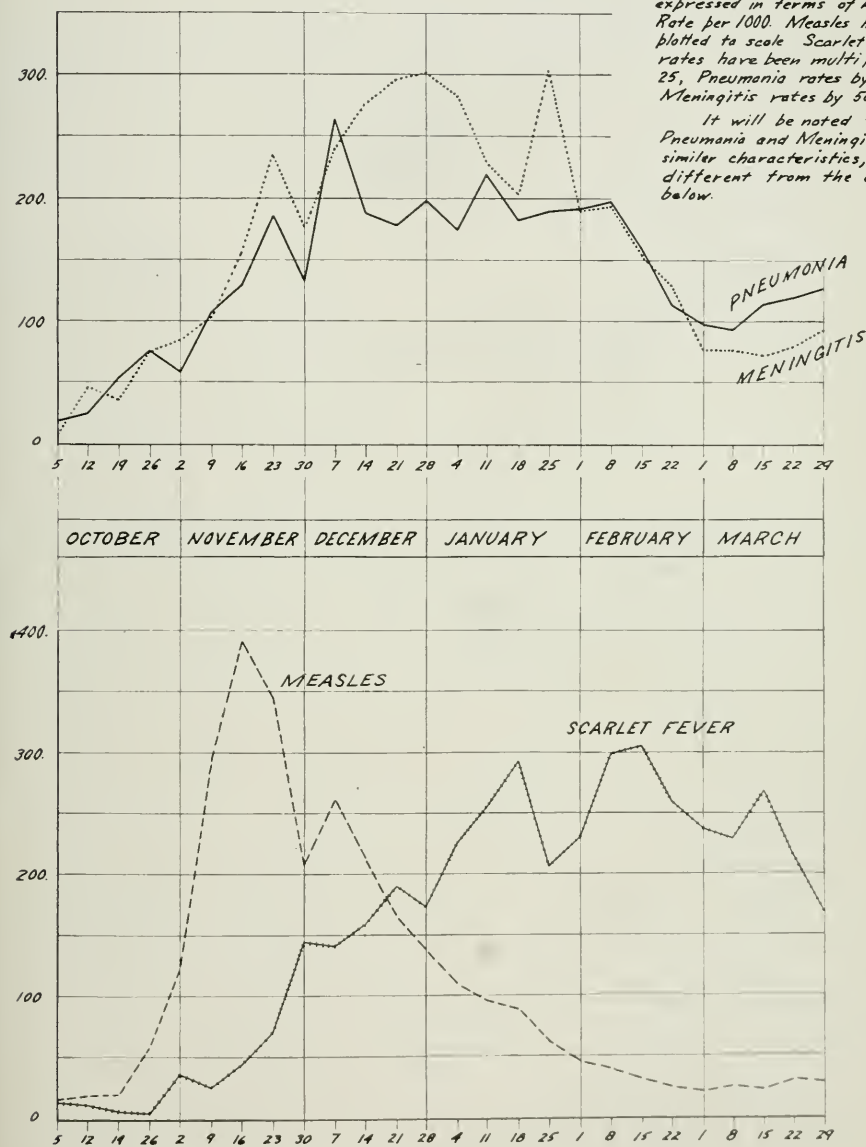


Chart XI.

the susceptibles or those who have not had measles who are attacked. Upon exhausting the susceptible material the disease disappears. This fact will be appreciated when we recite the experience at Camp Wheeler. (See Chart XXI.) From an annual morbidity rate of 2200 for the week ending Nov. 16, the rate dropped to zero on Jan. 11th, and with the exception of a few cases during three

weeks in February and March, there has not been a *single case of measles* at Wheeler as late as June 28th.

This is a remarkable illustration of how a disease such as measles can literally take a census of every susceptible individual in the camp and when finished, die out completely for want of further sustenance.

With measles it matters not as to the condition of health. The disease progresses as the infection is spread amongst the susceptible population.

With pneumonia and meningitis it is different. Tests have shown that many people carry the germs of these diseases and yet possess good health. It is not until the vital resistance is lowered sufficiently by fatigue or exposure that the disease germs present become activated and pass through the body's protective gates which have been opened to them.

Scarlet fever resembles pneumonia and meningitis in the curves more so than it does measles. This was epidemic in a few camps but was far less prevalent and less widespread than measles. Every camp had measles, pneumonia and meningitis. There were no cases of scarlet fever reported at two camps and the morbidity rate was less than 1.0 per 1000 in nine camps.

From the statistical analysis before us scarlet fever is less infectious than measles, is less widely disseminated, and has less susceptibles among the population.

#### TYPHOID AND PARATYPHOID

Typhoid fever and its associated disease paratyphoid have been of slight consequence in the American army during the past winter. But three camps showed it to any extent and even here the incidence was very low. Camps Dix, Bowie, and Sheridan had annual morbidity rates per 1000 of 2.1, 1.4 and 0.9, respectively. These figures are relatively high as compared with the following rates for people at all ages:

Kansas	1.3
Virginia	1.1
Virginia	.73
Massachusetts	.31
Michigan	.29

As over against this, however, there were 12 camps that *did not have a single case* of typhoid or paratyphoid. Included in this list are Wadsworth, Wheeler, Cody, Doniphan, Shelby, Beauregard, Kearny, Jackson, Sherman, Custer, Grant and Pike. Comparative death rates for the army and people of all ages in civilian life during the six winter months of the past year are given in Table 24.

This result would be even more striking if the civilian rates were computed merely for the age period 20 to 29 years, in which age group typhoid is most prevalent.

It would be difficult to put forward testimony of a more striking nature as to the efficacy of the preventive measures now being utilized in the army against typhoid fever. To be sure, the past season has not been the typhoid part of the year and it is possible that the value of the typhoid prophylaxis will be more severely tested during the warmer weather and the fly season.

The American soldier today has found the army camp a safer place to dwell, so far as typhoid fever is concerned, than in the most favorably situated civilian community. Chart XII has been inserted to show the decline in the typhoid death rate in the American army since 1897.

## TYPHOID FEVER

TABLE 24

TYPHOID AND PARATYPHOID IN ARMY AND CIVIL LIFE  
ANNUAL DEATH RATE PER 100,000

PLACE	RATE
All troops in U. S.	1.3
National Army Camps (16)	2.3
National Guard Camps (13)	.6
Massachusetts	4.1
New York City	3.2
Vermont	4.4
St. Louis	5.2
Connecticut	6.4
Colorado	10.
Michigan	11.
Virginia	18.
Maryland	18.
Kansas	22.
Kentucky	28.
*Delaware	55.

\*It is of passing interest that the National Army camp with the highest typhoid morbidity rate (Dix) is a mobilization point for Delaware troops.

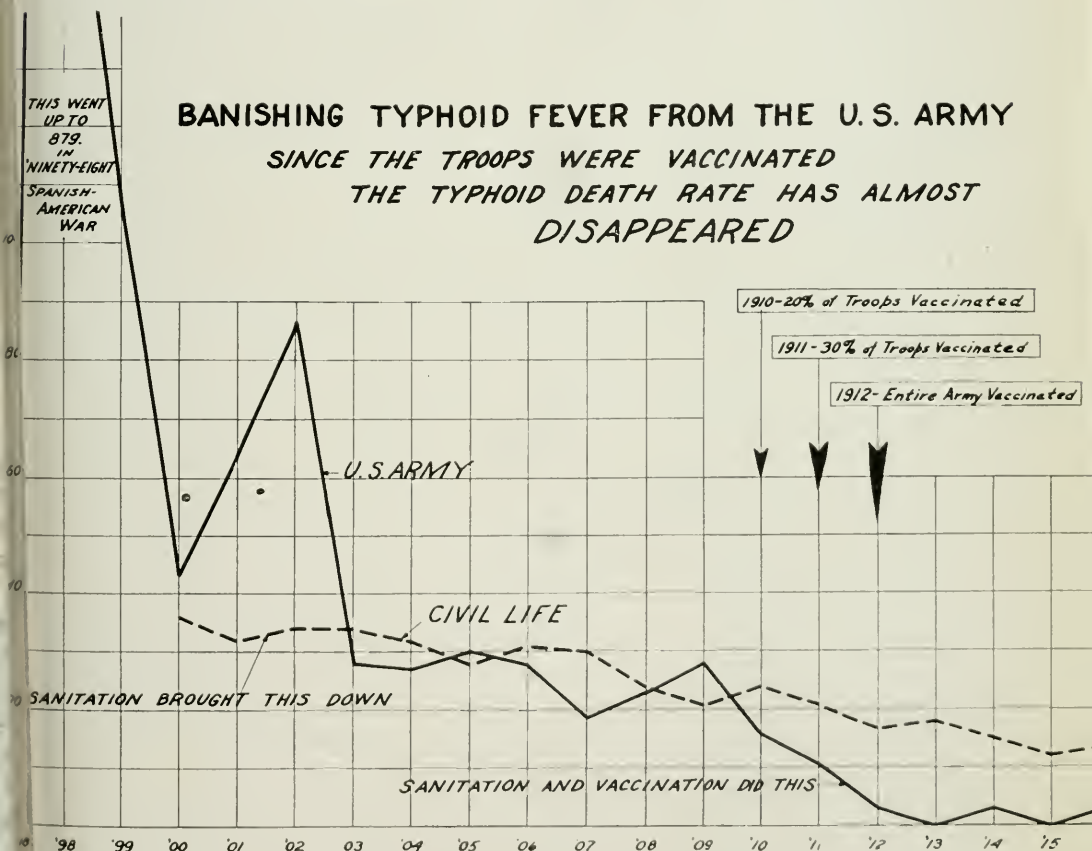


Chart XII.

## NOTE

Figures for Civil Life represent  
 U.S. Registration Area

## DIPHTHERIA

Deaths from diphtheria have occurred in only seven out of 29 camps, the rates for which are as follows:

(ANNUAL DEATH RATE PER 100,000)

Pike	30
Funston	22
Dodge	18
Cody	17
Custer	10
Doniphan	8
Logan	7

The death rate for diphtheria and croup in the U. S. Registration Area in 1915 for all ages was 15.7.

## TUBERCULOSIS

As illustrated in an earlier table the tuberculosis death rate in the Army is much lower than in civil life, owing to the elimination of the tuberculous by physical examination. Many recruits with undetected tuberculosis passed into the Army, however, and the deaths of some of these occurred within the past six months.

The tuberculosis death rate for the Guard and the National camps is given in Table 25:

TABLE 25

TUBERCULOSIS IN ARMY CAMPS  
ANNUAL DEATH RATE PER 100,000

CAMP	ARMY GROUP	RATE
Wheeler	N. G.	80
Pike	N. A.	44
Beauregard	N. G.	43
Wadsworth	N. G.	39
Sherman	N. A.	38
Lee	N. A.	32
Funston	N. A.	30
Kearny	N. A.	29
Upton	N. A.	27
Grant	N. A.	23
Jackson	N. A.	22
Gordon	N. A.	21
Custer	N. A.	20
Dix	N. A.	19
Sevier	N. G.	15
Dodge	N. A.	9
Sheridan	N. G.	9
McClellan	N. G.	8
Taylor	N. A.	8
Shelby	N. G.	8
Travis	N. A.	7
Hancock	N. G.	6
Lewis	N. A.	6
Logan	N. G.	0
Cody	N. G.	0
Doniphan	N. G.	0
Bowie	N. G.	0
Devens	N. A.	0
Meade	N. A.	0



## EPIDEMIC BRONCHITIS

This disease has prevailed at all camps, but the only detailed report we have had is from the epidemiologist at Fort Oglethorpe. In view of the fact that this and allied ailments, which are looked upon as of minor significance, are so widespread and so common to all camps and are so largely responsible for hospital and quarters cases of a few days' duration, each, it is felt that a description of the situation as reported from Oglethorpe is of especial interest. Illnesses of this character have assumed added importance since they have come to be regarded as predisposing factors to such diseases as pneumonia and meningitis.

Bronchitis has been well-nigh universal in the Oglethorpe group of camps. Within ten days after their arrival newcomers have generally been attacked, the symptoms often being pronounced from the start. Sneezing and coughing are early signs. In the barracks, mess halls, lecture rooms and places of amusement, during November, December and January there was seldom a moment when coughing was not noticeable and continuous. It is estimated that 80 per cent of all the troops were affected. The epidemic abated in February with the advent of warm weather. Laboratory findings do not agree that any single organism has been the cause. Streptococci, staphylococci, influenza bacilli and other organisms found in the noses and throats of healthy people and sometimes associated with disease have been isolated, but not under circumstances which have led to any of these germs being demonstrated as the microbic cause of the bronchitis. The infectious matter has passed from person to person, probably in three ways: First, men have talked with one another at close range, permitting mouth germs to be projected directly into one another's faces. In the second place, there has been a general impregnation of the atmosphere in confined spaces. In the third place, articles handled by the infected have been transmitted to others. In these ways the amount of infectious matter which has passed from person to person must have been large and meeting a lowered resistance, the infection has rapidly spread.

Predisposing and contributing causes existed to some extent. The weather was unfavorable. Changes in the temperature were frequent and marked. The ground was cold, the air damp and the nights cold. Often men did not have dry shoes for weeks at a time. Some spent the entire winter in khaki. The men themselves were ignorant of simple personal precautions which might greatly have lessened their chances of infection. All of the severe cases have gone to the hospital. The universal prevalence is significant both on its own account, and because of the light which it throws upon the spread of other respiratory diseases in these camps. Although most persons regard bronchitis with comparative indifference, it is a disease of much significance. It is the common saying among the troops that the "bronchial cold" which attacks them soon after their arrival remains with them as long as they stay in camp. Its characteristic hard, explosive cough remains after other symptoms have disappeared. For the time it often unfits a man for duty and there are few sufferers whose efficiency has not been impaired by it. The bronchial pneumonias of these camps have been frequent sequelae to bronchitis. Whether bronchitis renders a man especially susceptible to other acute respiratory diseases is a question of much interest. Like pneumonia one attack does not protect against others, but on the contrary

seems to predispose its victim to subsequent attacks. It is probable that bronchitis reduces resistance to measles, scarlet fever, pneumonia and other such diseases. The part which bronchitis may play in the spread of other respiratory infections gives reason for regarding it as a camp disease of the utmost importance. A carrier of meningitis may be relatively harmless so long as he is in good health, for then the germs which he harbors are fairly well locked up in his nose and in his throat, but when he experiences an attack of bronchitis he sneezes and coughs and at each paroxysm germs are shot into the air. Therefore, it is reasonable to suppose that measles, scarlet fever and other respiratory diseases have been spread by bronchitic soldiers. It may be asked if the exchange of bacteria from the nasal pharynx is so general, why is it that more sickness has not occurred. The answer probably is that most robust soldiers possess a fair degree of immunity acquired either by one attack as in the case of measles, or by frequent exposure without suffering from the disease.

The bronchitis which has prevailed in these camps may be designated as an acute infectious inflammation affecting in rapid succession downward the nasopharynx, the larynx, trachea, and first and second divisions of the bronchial mucosa. The symptoms are coryza, chilly sensations, hoarseness and soreness of throat, weakness, muscular soreness and slight fever (100 to 101 degrees). In severe cases the temperature may rise to 103 degrees with the correspondingly rapid pulse accompanied by substernal soreness and tightness of the chest. At first the cough was dry and unproductive. It frequently occurs in paroxysms causing muscular pain and soreness along the costal margin at the attachment of the diaphragm. On the third or fourth day, as a rule, the cough loosens and expectoration appears. At first it is scanty and mucous, later it is abundant and muco-purulent. Only negative results are obtained on palpation and percussion. On auscultation sibilant rales may be noted, except when resolution begins, when fine and coarse mucous rales are to be heard. The breath sounds are harsh. Bronchitis may be associated with other diseases such as measles, mumps or scarlet fever. When not complicated, recovery occurs at best within a week, although a chronic form may supervene and continue for months.

Although chronic bronchitis as seen in the quarantine camps is believed to be a definite entity, it is not always so diagnosed. Cough, elevation of temperature and muscular soreness accompany acute nasopharyngitis and tracheo-laryngitis as frequently as bronchitis. A positive diagnosis of bronchitis is impossible unless harshness of breath sounds, sibilant and sonorous rales are audible, or in a stage of resolution, fine and coarse mucous rales are heard. Many cases are diagnosed as bronchitis from the symptoms alone.

Pneumonia is not frequently preceded by acute bronchitis, and acute bronchitis rarely terminates in pneumonia except when it accompanies measles. In measles there may be an extension to the terminal bronchi, and the intercommunicating air cells, under which circumstances bronchopneumonia develops.

#### INFLUENZA

Influenza, like bronchitis, has prevailed widely and we are indebted again to the epidemiologist at Oglethorpe for a description of this disease which undoubtedly describes the situation at many camps.

A disease strongly resembling influenza became prevalent in the Oglethorpe camps about March 18, 1918. It soon assumed endemic proportions. Within

two weeks every organization in Camp Forrest and the Reserve Officers' Training Camp was affected. It seems to have visited only a part of Camp Greenleaf. The War Prison barracks were not invaded. After about three weeks the epidemic subsided rapidly. The number of cases sent to hospital or to quarters was 1468 in a total strength of 28,586. Owing to the fact that many cases were not severe, the total number of officers and men attended can not be given; an estimate based on replies to a circular letter of inquiry to the several organizations, indicates that not less than 2,900 cases have occurred in Chickamauga Park.

The attention of the Camp Surgeon's Office was called to the existence of this disease on March 18th at which time the writer saw a number of men appear at sick call in the 51st Infantry, suffering with the disease which the regimental surgeons were unable to diagnose. The symptoms were as follows: Headache, pain in the bones and muscles, especially the muscles of the back, marked prostration, fever (sometimes as high as 104). Sometimes there was conjunctivitis, coryza, a rash and possibly nausea, recovery taking place in a few days.

In most cases a definite diagnosis was not made at the regimental sick call, but at the receiving ward, when a name was given, it was usually called influenza.

On April 3rd it was recommended that steps be taken to ascertain from the several organizations under the supervision of the camp surgeon and from other camps in the Oglethorpe region, the essential facts concerning the nature and epidemiological progress of the disease. A circular letter of inquiry upon these lines was sent out on April 4th to all organizations. Replies to this questionnaire indicated that the disease was first noticed in epidemic form on March 18th in the 51st Inf. In some organizations all cases were sent to hospital; in others their cases were treated in quarters or in the regimental infirmary. This does not mean that the disease was more severe in some organizations than in others. Difference in the disposition of the sick depended not so much upon the severity of the case as upon the local facilities for dealing with them. Records show that there were twice as many patients dealt with in quarters as in hospitals and there were probably twice as many cases existing as were carried on sick report.

The replies were unanimous in stating that the disease was not restricted to recruits nor to men who had already experienced or failed to experience an attack of measles, German measles, or scarlet fever. One attack did not protect against another.

In all the organizations the epidemic was first located in companies before it became general. In many instances a large proportion of the men were affected. According to the registrar of the Base Hospital, fully one-half of the total number of patients in the hospital had the disease. The rate at which the epidemic progressed made it impossible to trace its path, if indeed it followed any.

The incubation period was short, usually not over one or two days. Instances were found in which men isolated in quarantine were attacked apparently through contact with those who brought them food or approached them for other reasons. At the Post Hospital, a number of surgical cases in tents were infected, apparently by an orderly. In Co. B, of the 15th Machine Gun Battalion,

which was in quarantine on account of scarlet fever, nearly every one present was attacked. In Company A, 52d Infantry, a company which was quarantined, the men appeared to be protected by reason of their isolation.

Some organizations suffered more than others for no apparent reason. The 52d Infantry had the most and the 54th Infantry the fewest cases. In the 52d Infantry and the 15th Machine Gun Battalion, one in every seven of the officers and men were on sick report. In one ambulance company the proportion was one to six, while in others it ran as low as one to twenty-five.

The possibility that the disease which has just become epidemic has long existed in these camps in sporadic form, but has not hitherto attracted notice because of its infrequency, has received careful consideration. At first there seemed much to recommend this theory. Clinicians on the hospital staff claimed to have seen the disease at times since the summer of 1917. They say that it became rather prevalent in September. It is believed by some that the germ of influenza, like that of some other diseases, is constantly present in every large community; that it is not always virulent, but under certain conditions it acquires increased virulency.

If the organism which has caused the present epidemic has long been in existence in the Oglethorpe camps, it is not apparent why it has suddenly become so active. It would seem that the conditions for its epidemic prevalence have existed for a long time. Recent weather conditions, uncomfortable as they have been, have not been so disagreeable as they were in January. The theory that the explosion is of local origin needs a better explanation than can apparently be given it.

The weather was cloudy, damp and chilly. It was not cold nor wet, but the nights and especially the mornings were decidedly damp and uncomfortable. The difference between the air indoors and out was marked. Drafts were particularly noticeable.

Reviewing these facts about the weather, it can not be said with certainty that the conditions of temperature and humidity have had much to do with the epidemic, nor can it be denied that they played an important part in predisposing the troops to attack. Obviously the weather conditions have led the men to gather together indoors where they have been especially exposed to infection from one another, and it has had a chilling effect which is an important factor in the progress of all respiratory infections.

An inquiry was made to ascertain whether the troops were exposed to an unusual extent to the weather, to excessive fatigue, or in any other way before or after the epidemic started. This line of investigation brought no suggestive information to light.

No exceptional prevalence of influenza or other similar infectious disease has existed in Chattanooga or in the extra cantonment zone. An effort was made to shed light on the identity of the disease by studying the record of other cases of sickness which had been sent to the hospital under the designations of "fever, type undetermined" and "influenza" during the six months preceding the epidemic. These designations have never been definite; they have seldom been based on conclusive evidence at the hospital. Bacteriological examinations have seldom proved the influenza bacillus to be the causative agent of the cases



called "influenza." From time to time this bacillus had been found in the secretions of the nose and throat of troops, but has not been proved to be the cause in any large amount of sickness. "Fever, type undetermined" and "influenza" have been merely convenient expressions by which to designate cases of an indeterminate sort which demanded treatment and had to be called something.

Of a series of 161 cases of "fever, type undetermined" sent to the hospital before the present epidemic broke out, 59 turned out to be bronchitis and 41 pneumonia; 61 were called "influenza." In some instances "fever, type undetermined" had been found to be measles, scarlet fever, otitis media and meningitis. Out of 189 cases diagnosed "influenza" at the regiments, 150 turned out at the hospital to be some other disease.

It is probable that the epidemic disease was recently brought to these camps. If it is genuine influenza, and the epidemiological features no less than the leading symptoms seem to point to that disease, there is here offered the most reasonable explanation of the outbreak which is now possible. No other disease spreads so fast or is so prostrating, considering its symptoms. Influenza may be nearly explosive in character. It spreads as rapidly as personal communication permits. Personal contact is intimate in the Oglethorpe camps, especially between men in companies and regiments. To some extent the regiments keep separate, but there is a general mixing at places of amusement in camp and Chattanooga. It is worthy of remark that the regular Officers' Training Camp is an organization which mingles but little with others in the Oglethorpe camps, and that here the epidemic was late in appearance. The same may be said of a part of Camp Greenleaf. The War Prison Camp is entirely separate and escaped infection. The epidemic seems to have burned out for want of suitable material, probably with the gradual but rapid decrease in virulence.

Reviewing the whole subject, it may be said that an epidemic of influenzal disease became prevalent in the Oglethorpe camps toward the latter part of March, 1918. The identity of the disease has not been positively determined after nearly a month of observation. It may have been an outburst of a form of sickness which has long existed in sporadic form in these camps. It may have been brought to the camps from outside. The weight of evidence is in favor of the latter theory.

It is a highly infectious disease with a short period of incubation. The weather has encouraged the epidemic, but is not apparently responsible. The disease is respiratory in type, with a strong resemblance to influenza in some of its most characteristic symptoms, as note the fever, pain in the back and legs and great prostration.

The cause of the rapid spread undoubtedly lies in the great infectivity of the causative agent, its short period of incubation and the intermingling of the troops. One thing seems clear, the disease could never spread unless the buccal or nasal discharge of the sick got into the mouths or noses of susceptible persons. The nature of the epidemic seems to show the extent to which this interchange takes place under the conditions which surround these troops.

#### ANALYSIS OF CAUSES OF DISEASE IN THE ARMY

In the preceding pages we have set forth the primary data of disease incidence in the army as a whole and for certain National Guard and National Army

camps. It has been pointed out that respiratory disease has prevailed to a much greater extent in the army than in civilian life. In the army 77 per cent of all deaths were caused by this group of six diseases during the period covered. In civilian life but 43 per cent of all deaths in a comparable age group are due to respiratory disease.

The most important of these six diseases is pneumonia in that about 80 per cent of the deaths from the respiratory group are attributable to this one cause. Meningitis is second in importance in being responsible for 15 per cent of the deaths in the respiratory group. This leaves but 5 per cent of the group to be accounted for by the four other diseases—tuberculosis, measles, scarlet fever and diphtheria.

Pneumonia and meningitis are important not only because they cause so many deaths, but because the excess of these two diseases over that in civil life is more pronounced than the excess of other diseases in the army over their extent in civil life. In the calculation made, meningitis was seen to be 45 times greater in the army, pneumonia 12 times greater. Measles was 19 times greater in the army but this is not of as great significance as the other two as the outcome is less fatal. Scarlet fever and diphtheria are only slightly more prevalent in the army than in civil life. It is the underlying cause of this excess disease incidence in army camps that we are particularly interested to discover.

We have noted that there is a wide variance in the rates at the different camps and although the average for all troops is raised by excessive rates in a few camps, the majority of the camps still have rates appreciably above the rate for civil life. Certain influences seem to be acting on all camps and certain other influences are confined to a certain group of camps. We may classify these influences under three main heads. The prevalence of disease may be attributed to one or more of these causes:

1. Weakening of the resistance of the soldier due to
  - (a) Exposure to severe weather.
  - (b) Insufficient clothing.
  - (c) Inadequate housing, lack of heat.
  - (d) Fatigue.
2. Unusual facilities for the transmission of the infective agent by—
  - (a) Close contact with carrier cases.
  - (b) Undetected cases among new recruits.
  - (c) Importation of mildly sick men and carriers from other camps.
  - (d) Association with civilian community.
  - (e) Overcrowded quarters.
  - (f) Inadequate hospital care of patients.
  - (g) Unsanitary conditions in general.
3. Natural susceptibility to disease.
  - (a) Racial influence.
  - (b) Effect of rural life.
  - (c) Climatic influence.

## 1. INFLUENCE OF THOSE FACTORS WHICH BRING ON PHYSICAL DEBILITY

Under this heading we may consider the influence of those factors which lower the physical tone of the body and make the natural defenses of the body less resistant. The recruit fresh from civilian life undergoes a considerable change in his mode of living on entering camp. There he becomes a part of a vast machine. He has less opportunity to cater to his personal wants. He is out of doors more. He is apt to become chilled or wet. He sleeps in colder quarters, and not only sleeps but lives, eats, undresses and dresses, bathes, etc., in colder quarters. Being out of doors more his appetite is greater. He eats more heartily. He is inclined to overeat. In his desire to get ahead in competition with his associates he is apt to work harder, to become unduly fatigued. When indoors, in an effort to keep warm, he joins the crowd about the stove. He may be subjected at times to overheated atmospheres.

Each and all of these influences tend to pull down the physical resistance of the recruit. They put a strain upon him *and especially so upon the man who hasn't been used to this sort of thing*. It is the untrained individual who feels the rigors of camp life most keenly.

With a realization of this state of things we may consider to what extent these factors have acted at the different camps. First we are confronted with the facts so strikingly illustrated in the charts, namely—

The morbidity rate from pneumonia is *greatest* in Camps: Bowie, Wheeler, Travis, Pike and Cody.

The death rate from all causes is *greatest* in Camps: Pike, Wheeler, Beauregard, Bowie and Jackson.

The morbidity rate from meningitis is *greatest* in Camps: Jackson, Beauregard, Funston, Doniphan and Bowie.

The morbidity rate from measles is *greatest* in Camps: Pike, Bowie, Wheeler, Sevier and Beauregard.

The death rates from all causes are *lowest* at Camps: Sheridan, Hancock, Wadsworth, McClellan and Logan.

The morbidity rates from pneumonia are *lowest* at Camps: Sheridan, Wadsworth, Dix, Custer and Hancock.

The morbidity rates from meningitis are *lowest* at Camps: Wadsworth, Logan, Devens, Dix and Upton.

The morbidity rates from measles are *lowest* at Camps: Dix, Lewis, Hancock, Wadsworth and Upton.

In the face of the above we should expect, that if exposure were a factor in increased disease incidence, that the exposure was the same at each of the camps where disease was prevalent, and that this exposure was entirely different from that at the camps where disease was rare. We may examine this point further.

(a) *Weather*.—The weather of the past winter has been unique in some respects. East of the Rocky Mountains, the temperature in general has been the coldest in 46 years. West of the Rockies it has been the warmest in 46 years.

At Macon, Ga., October, 1917, was the third coldest October and January, 1918, was the coldest January in 46 years. January was an unusually cold month at San Antonio, Texas. October, 1917, was the coldest and January, 1918, the

third coldest in Chicago for 46 years. December, 1917, and January, 1918, were the coldest New York City has seen for 46 years.

At Los Angeles, Cal., the year 1917 witnessed the warmest December in 40 years and this holds true for Walla Walla, Washington. Weather data are reported graphically in Chart XIII.

If the low temperature had been a prime factor in the cause of disease we should expect the northern camps to suffer most, the southern camps next and

## MEAN OUTDOOR TEMPERATURE DURING THE WINTER OF 1917-18 AS COMPARED WITH AVERAGES OF PAST 32 TO 46 YEARS

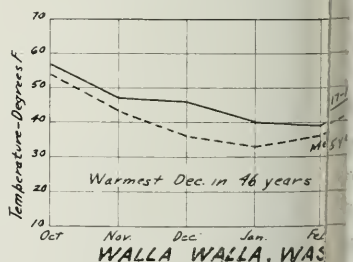
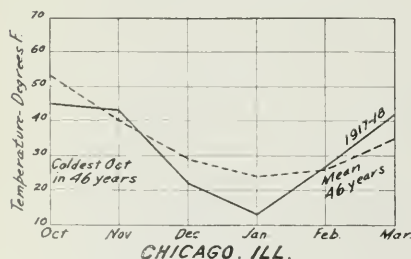
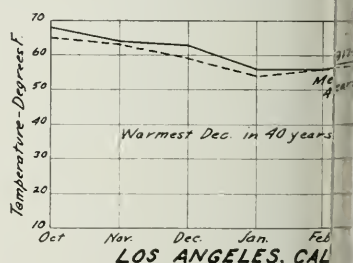
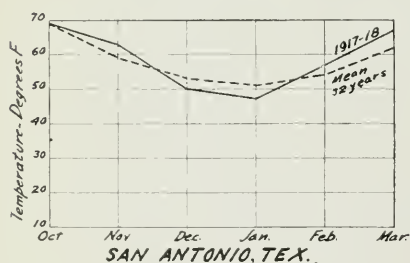
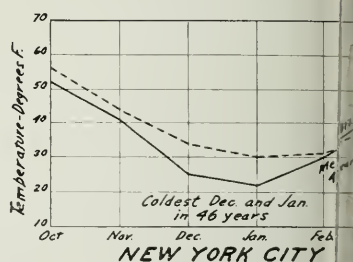
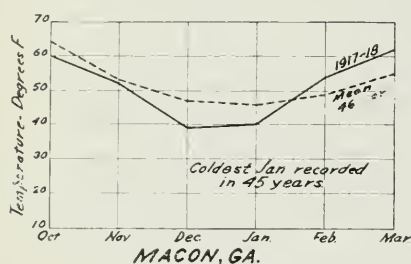


Chart XIII.

the far western camps, Kearny and Lewis where the weather was warmer than usual, least. This is not the case by any means. Camps Devens and Upton are probably the coldest camps and yet their disease incidence has been among the lowest.

The possibility for undue exposure to weather and its consequences is suggested in the reports from Camp Travis. Speaking of pneumonia a report reads that "the majority of the patients stated that their colds commenced after being chilled while on guard or at drill or when cold during the night and



that these colds commenced at or about the dates of 'Northers' and a sudden drop of temperature. The exposure and chilling is rather significant as a predisposing cause of pneumonia in this camp. It will be noted that no cases occurred in the Quartermaster Corps, Ordnance Corps, Base Hospital personnel, Bakers' Company, and the Bakers' and Cooks' School. Neither have any of the 1758 officers of this camp had pneumonia. These organizations are not required to do drill or guard duty. Their duty is mostly indoors, while other organizations are exposed to the cold, wind and dust. With the exception of the above named organizations, pneumonia is widely distributed throughout the camp."

Reports from Camp Beauregard state that there was a direct relation between the epidemic of pneumonia and the severity of the weather, the epidemic subsiding with improvement of the weather.

Severe weather undoubtedly caused trouble in some camps but this was not the controlling factor by any means.

Camps Bowie, Beauregard and Logan experienced about the same climatic conditions. The annual pneumonia morbidity rates per thousand for the first two were 96. and 42. respectively. That for Logan was 16.

Camp Pike is in about the same latitude as Camp McClellan. The pneumonia rate for the former is 63., that for the latter 9.6.

Camp Wheeler and Camp Sheridan faced the same weather. The pneumonia rate for Wheeler was 95., that for Sheridan 9.3.

Camp Jackson is close to Camps Wadsworth and Hancock. The former is high in the disease table, the latter low.

We are aware of the fact that we are leaving this question of the relation of the weather to the acute respiratory diseases and especially to pneumonia in a very unsatisfactory state. There are strong reasons for believing that the incidence of pneumonia is greatly influenced by weather conditions. Curves showing weather conditions as influencing pneumonia, in some of the camps, are striking and this evidence comes from camps as far apart as Sevier and Upton. We hope that more attention will be given to this matter in future studies on the epidemiology of pneumonia.

(b) *Insufficient clothing*.—Associated with the question of weather severity is that of inadequate clothing. The severity of the winter is felt less if the men are provided with plenty of warm clothing. There are a number of instances where the lack of winter clothing was felt, specific mention of this being made at Camps Sevier, Wheeler, Logan, Bowie, Sheridan, Shelby, Beauregard, Kearny, Devens, Dix, Lee, Jackson, Custer, Pike, Dodge, Funston and Travis. This factor without doubt increased colds which may in turn have led to pneumonia. For instance at Camp Jackson as late as January it was reported that "many men \* \* \* \* are seen going about in cotton clothing, despite freezing weather." At Pike we note the following from the report of an inspector: "The clothing of all the men in November and December according to the records was not complete. Many men were undoubtedly exposed to changes in temperature which caused a lowering of their resistance thereby making the spread of infectious diseases much easier."

The onset of measles and pneumonia at Camp Sevier was attributed to

exposure to weather and lack of clothing. The report reads—"During the latter part of October a sudden drop in temperature occurred causing considerable discomfort of the troops, many of whom were poorly clad. It was at this time a large increment of draft men was received. Many of these recruits had been exposed to measles; they were poorly nourished. The sudden exposure of these troops in this condition resulted in many cases of pneumonia, many deaths occurring."

Judging from this, Jackson, Pike and Sevier suffered from lack of clothing. These camps have high disease incidence. But clothing shortage was by no means peculiar to those camps with high disease incidence. Referring to the previous list we note that Logan, Sheridan, Devens and Dix are also included and yet these camps had relatively little sickness. In November at Sheridan "few men had woolen underclothing or overcoats" and men could not sleep at night because the two blankets provided were insufficient to keep them warm. At Devens as late as December we learn from the report of inspectors that the clothing of men was insufficient and that "there is much suffering from cold and many cases of frozen ears and fingers are incurred by men on duty in weather below zero without winter gloves and ear protectors."

Lack of clothing was a contributing factor in disease incidence but it is not believed that this cause was responsible for the peculiar distribution of diseases in the different camps.

(c) *Inadequate housing and lack of heat.*—In addition to lack of warm clothing there was also felt the lack of warm quarters. It is aggravating and debilitating to be cold when out of doors at work, but it is even more serious when there is no opportunity to retire for a time to warm quarters. In some instances lack of fuel made it impossible for men to keep warm indoors during day and night. But here again there is no evidence that Pike, Beauregard, Wheeler and similar camps suffered more from this cause than Logan, Wadsworth, Devens or Upton. If anything, the northern camps would suffer more in this respect and yet the northern camps had less disease. Similarly if lack of fuel and cold quarters were a controlling factor we should expect the National Guard camps to show more disease because of their tent quarters, but this is not the case.

(d) *Fatigue.*—It is well known that overwork leading to excessive fatigue lessens resistance to disease. In view of the previous life of many recruits who have come from offices and factories, it is probable that the vigorous outdoor life of the camp introduced suddenly pulled down the general body resistance. Here again, however, we must point out that the records at hand do not show that fatigue was any more of a factor at Pike, Beauregard, Bowie and allied camps than at Logan, Devens, Sheridan and others of this type.

The report from Wheeler is that "fatigue has not been a factor" in disease incidence. From Wadsworth we are informed that "fatigue has been a contributing factor in the etiology of respiratory diseases, but a very minor one. Essentially the same conclusions are obtained from those familiar with the situation at Lee and Lewis.

The Division Sanitary Inspector at Travis believes "that men upon entering military service are subjected to a too strenuous program of drills for the first four weeks. It would be better if the first four weeks of military service were

given over to light exercise and short periods of drill which could be slowly increased. This would give the men a chance to better accommodate themselves to the new life and the body a chance to build up physical resistance against disease."

In general fatigue has evidently made troops somewhat more susceptible to disease, but this has been a general tendency and not one peculiar to any particular group of camps.

## 2. INFLUENCE ON DISEASE INCIDENCE OF THE UNUSUAL FACILITIES FOR THE TRANSMISSION OF THE INFECTIVE AGENT, AFFORDED BY CAMP LIFE

(a) *Close contact with carrier cases.*—The bringing together of men from all sections into camps naturally increases the possibility of contact transmission of disease. It will be readily appreciated that a carrier of the meningococcus can do more damage in an army camp of 30,000 than he could on a farm where his association is limited to few individuals. The prevalence of sickness in the various camps has without question been augmented by the presence of individuals who are themselves well but who carry in their noses and throats the germs of the respiratory diseases, but there is no reason for believing that the number of such carrier cases is so much greater among the men of Camps Pike, Beauregard, Wheeler, Bowie, Jackson than among the men of Camps Logan, Hancock, Sheridan, Wadsworth or McClellan. In other words there is no indication that the germs of respiratory disease are any more widespread to begin with in the former camps than the latter. This probably is not true of meningitis. It is certain that the areas from which Jackson and Funston drew men are those in which this disease has been endemic.

To check up this point we need statistics on the number of carriers among the civilian population in these various districts and this material is lacking.

The importance of the carrier is a mooted question. In view of the great number of carriers of certain diseases which have been discovered it is a question whether or not it is practicable to even attempt their detection and eradication. It seems more likely that immunity or physical resistance is more potent a protective force than efforts toward keeping away from the germ itself.

There were found at Camp Travis, during January, 112 positive meningococcus throats among 3,159 men examined. This amounts to 3.5 per cent of positive carriers. This number of carriers in one month is nearly three times the number of actual cases of meningitis reported from Travis for the entire six months.

At Camp Jackson 8 per cent of all hospital admissions were found to have positive meningococcic throats. As this is a higher percentage than is found among the population at large, it is believed by the epidemiologist at Jackson that lowering of the physical tone renders one more receptive to the meningococcus.

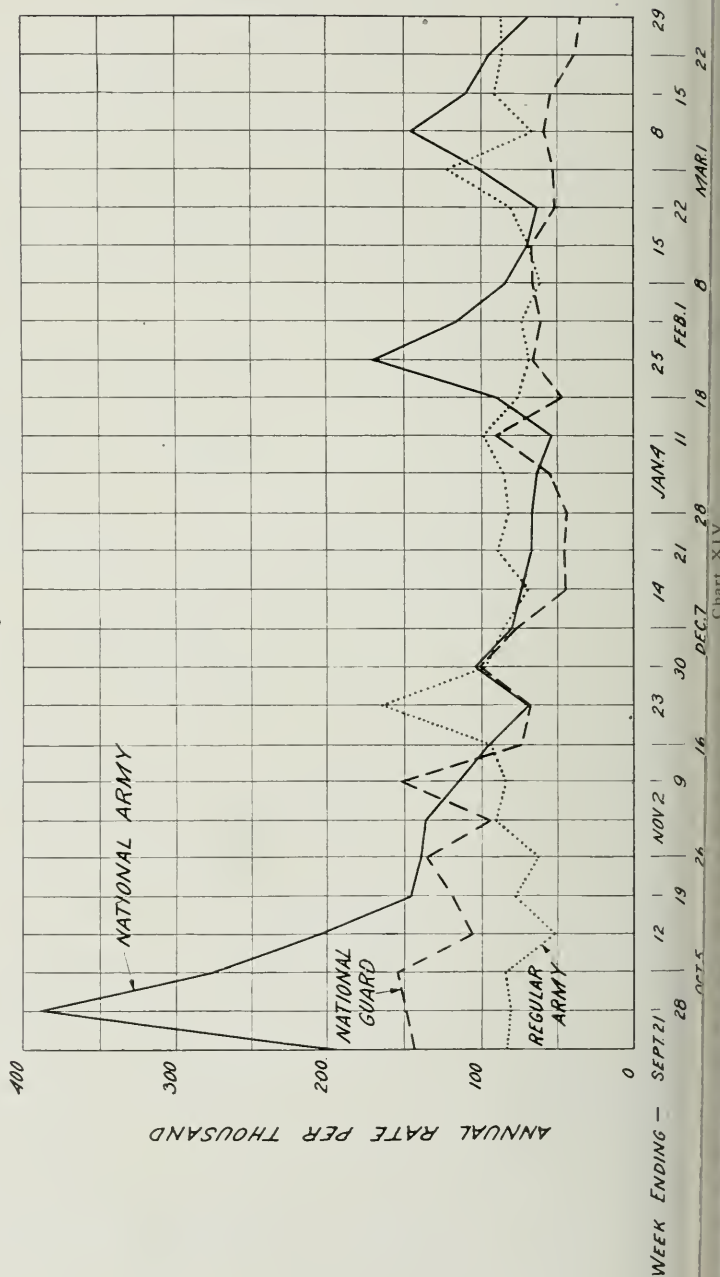
At Camp Bowie, during February, seven cases developed among 114 carriers. If the persons coming in contact with this number of carriers had not possessed some immunity to this disease the cases would have been much more numerous.

It is reported from Camp Grant that diphtheria carriers accumulated there at one time to the number of one hundred. They were quartered at the Base Hospital while continued unsuccessful efforts were made to clear up their throats. Finally in desperation almost all of these men were turned loose and returned to duty with their companies and no increase in diphtheria occurred as a result.

# VENEREAL DISEASE IN THE THREE GROUPS OF THE U.S. ARMY WEEKLY INCIDENCE EXPRESSED IN TERMS OF ANNUAL CASE RATE PER 1000

SEPT. 1917 TO MAR. 1918 INCL.

NOTE: The influence of the new recruit  
is suggested by the high rate for the  
National Army.





(b) *Undetected cases among new recruits.*—In a sense this matter has already been covered in the previous discussion. There is a new problem introduced here, however, in that men fresh from civilian life are likely to act as foci of infection.

Pneumonia, measles and meningitis are to be found widely spread over a state. There is scarcely a county wholly free from these diseases at any one time. The gathering of the drafted men brings in many who are either coming down with the disease or who are carriers.

Reports from Wadsworth state that "new troops shortly after arriving in this camp generally showed quite a large number of sore throats. These would disappear after the troops had been in camp for several weeks."

Reference has already been made to the onset of measles and pneumonia at Sevier coincident with the coming of new draft men. Sevier "was practically free of all communicable diseases until the influx of the draft men from Camp Jackson; our first measles case appeared at this time, in the early part of October. Scarlet fever did not appear in this camp until troops arrived from Jefferson Barracks, Missouri."

The Division Surgeon at Camp Wheeler in a published report describing the epidemic of measles and pneumonia there, states that "draft men brought measles on every train." "Six cases were taken from one train."

This is especially noticeable in the case of venereal disease. The admission rate from this cause went up enormously in the National Army with the accession of new men. As time went on this fell off until the rate was no higher than that for the Guard or Regular Army.

This fact is graphically shown in Chart XIV.

New men increase disease in camp but this cause should act no differently in one part of the country than another.

(c) *Importation of mildly sick men and carriers from other camps.*—More striking than the association of disease with new recruits has been the association with importations from other camps. There has apparently been an endeavor on the part of some to weed out the undesirables and ship them along when the call for transfer has come. This has been complained of at many camps and that there are just grounds for this complaint is evidenced by the many instances of sick men being found among troop arrivals. This is an influence which would react differently on the camps. Thus, if one camp is gradually being recruited in strength and not shipping men away it may at the same time accumulate an abnormal proportion of physical weaklings. Or a camp may act as a mobilization center both receiving and shipping troops. If there are sick men among the arrivals these are filtered out and an undue accumulation of hospital admissions will take place sending the rate up above that of a relatively closed camp. It is felt that Funston and Dodge have suffered notably in this respect.

An examination of how this works may be taken from the experience at Camp Sheridan occupied almost entirely by Ohio National Guardsmen. In April a contingent of over 700 men was received from Camp Travis. Immediately on obtaining a physical examination of this group was made and revealed the following:

Venereal disease	40 cases
Mumps	3 "
Measles	8 "
Pneumonia	2 "
Meningitis carriers	27 "
Trachoma	2 "

These cases should have been discovered before this contingent left Texas and the infected persons isolated from the rest. By reason of the failure to do this the infection spread while in transit and there appeared shortly after in addition to the cases examined on detraining the following:

Venereal disease	8 cases
Mumps	37 "
Measles	6 "
Diphtheria	2 "
Pneumonia	8 "
Meningitis carriers	40 "

Camp Beauregard, speaking of the meningitis and measles there, states, that the epidemic started with the arrival of troops from Camp Pike and the State mobilization camp of Mississippi.

Camp Pike on the other hand transmits the blame to other camps—"A study of the character of material received from other camps shows that in many instances physical derelicts were sent to Camp Pike." Contributing to Pike were troops from Custer, Funston, Grant, Sherman, Taylor and Dodge.

Reports from Camp Wadsworth state that "among those troops received from Camps Taylor, McClellan and Fort Oglethorpe, one case of meningitis and one case of measles were taken from the train on which the troops arrived."

Speaking of the new increments from Camp Taylor this report goes on to say that the noneffective rate for this new group went up to about 79 per 1,000 whereas the noneffective rate for the division previously was around 24.

Introduction of scarlet fever, measles and mumps are all attributed to importation of men from other camps by the epidemiologist at Camp Upton. These diseases were present among a small increment from Camp Dodge. On distributing these men about the camp these diseases began to appear in those organizations to which they had been assigned.

Camp Cody attributes the entrance of epidemic disease to new accessions. Thus "measles and German measles . . . followed shortly after the arrival of troops from Camp Dodge."

The epidemiologist at Custer reports that many of the new arrivals entered camp suffering from acute respiratory infections or promptly developed them. "The succeeding incidence of pneumonia was principally among the unseasoned troops."

(d) *Infection from the surrounding civilian community.*—Disease has been introduced into camps from the civilian community. This may occur in camps long established and among troops who have been in the service for some time.

Data from Camp Upton is interesting in this connection. Five cases of measles occurred in the 304th Field Artillery. The first man had been home in Philadelphia two weeks previous. The second man had been home in Sussex, N. J., 16 days previous to his admission to the hospital. The third man sent to the hospital on April 16th had been home in Olean, N. Y., from April 3rd

6th. Data is incomplete in the other two cases. This evidence points to infection from civil life.

The epidemiologist at Camp Beauregard states that meningitis was prevalent in the civilian community around the camp and was responsible for its introduction into the camp. This was also true for measles.

Transmission of scarlet fever in this manner has been discussed at length on page 669.

(e) *Overcrowding*.—The early mobilization of the National Guard troops resulted in a great amount of overcrowding. There are instances of ten or more men to a tent that accommodates comfortably not more than five or six. This herding together of the men permitted unusual opportunity for transmission of the disease from one person to the other by contact, sneezing and coughing, and infected dust.

Specific instructions were issued to provide quarters which would give each soldier at least 500 cubic feet of air space. Just how serious this crowding was as a factor in spreading disease is a question. That it was responsible to a certain degree there is little doubt, but it is a very significant fact that there were remarkably few instances of more than one case of pneumonia or meningitis in the same tent. If this unusually intimate association of the men had been a controlling factor we would expect not one but four, six and eight cases to a tent.

In this connection it must be pointed out also that overcrowding occurred in those camps with little disease incidence as well as in those with high disease incidence. Crowding was by no means confined to the camps with excessive sickness.

A special inspection made at Camp Wadsworth in January showed instances of 9 men to a tent. There should not have been more than five in order to allow 100 square feet of floor space per person. This camp had a relatively low disease incidence.

Camp Logan had but 28 square feet of floor space per person for a long while. Camp Lee had as many as 15 men to a tent as late as November. Excessive sickness in certain of the southern camps can not be charged to this cause.

On the other hand, while crowding is not a controlling factor, it has been undoubtedly an incidental factor as evidenced by the difference in disease incidence between officers and men, the latter being brought into much more intimate contact with each other than the former. (See Table 26.)

In morbidity from measles the enlisted men exceeded the officers by appreciable proportions in every one of the 11 camps. Pneumonia morbidity has been greater among the men in 9 camps. Meningitis and scarlet fever were more prevalent among the men in all except one instance, meningitis at Beauregard being greater among officers. Deaths from these diseases were so few among the officers as to make any comparison of rates uncertain. Accepting the figures as they are, however, we find that deaths among the men were greater 27 out of 44 cases.

It is of interest to note that at Wadsworth, Grant, Custer, Meade and Travis the excess of measles was about 2 or 3 times greater among the men. At like, Beauregard, Lee and Jackson the ratio is nearer 10 to 1. It would appear

TABLE 26

COMPARATIVE MORBIDITY AND MORTALITY AMONG OFFICERS AND ENLISTED MEN  
SIX MONTHS, SEPTEMBER 29, 1917, TO MARCH 29, 1918

FIGURES IN ANNUAL RATE PER 1000

CAMP	MEASLES				PNEUMONIA			
	CASES		DEATHS		CASES		DEATHS	
	OFFICERS	MEN	OFFICERS	MEN	OFFICERS	MEN	OFFICERS	MEN
Wadsworth	2.2	7.9	.0	.0	4.3	9.1	2.2	1.1
Dix	4.8	34.	.0	.0	9.5	8.0	3.6	1.5
Grant	26.	50.	.0	.08	4.	14.	2.0	1.5
Custer	21.	65.	.0	.49	8.2	7.0	2.7	1.5
Meade	25.	36.	1.1	.0	3.2	18.	2.2	2.6
Lee	5.9	85.	.0	.19	18.	24.	9.8	5.5
Gordon	33.	168.	.0	.21	4.	15.	1.3	5.3
Jackson	36.	230.	.0	.33	5.9	36.	.0	11.
Travis	66.	*124.	.0	.0	7.1	78.	1.2	11.
Pike	35.	390.	.0	.15	32.	63.	1.3	25.
Beauregard	28.	256.	.0	.11	21.	42.	.0	15.

CAMP	MENINGITIS				SCARLET FEVER			
	CASES		DEATHS		CASES		DEATHS	
	OFFICERS	MEN	OFFICERS	MEN	OFFICERS	MEN	OFFICERS	MEN
Wadsworth	.0	.8	.0	.20	.0	.8	.0	.0
Dix	.0	.6	.0	.19	2.4	6.4	.0	.09
Grant	1.0	1.3	1.0	.31	6.0	12.	.0	.23
Custer	.0	1.8	.0	.30	.0	6.5	.0	.20
Meade	1.1	2.4	.0	.66	4.2	7.7	.0	.07
Lee	2.0	2.7	2.0	1.2	.0	.8	.0	.0
Gordon	1.3	3.6	.0	.96	.0	.1	.0	.0
Jackson	5.9	26.	1.2	7.5	.0	.9	.0	.0
Travis	1.2	2.7	1.2	2.5	.0	.4	.0	.0
Pike	.0	3.8	.0	2.4	7.9	44.6	.0	.44
Beauregard	28.	12.8	19.	7.7	.0	.0	.0	.0

\*Many cases not reported.

from this that the men at the latter camps are much more susceptible to the disease. There is no such big divergence between officers and men for pneumonia. Meningitis shows still less difference between officers and men. Scarlet fever is somewhat too irregular to summarize. One interpretation offered for the peculiar characteristics of the first three diseases is that measles is much more readily transmitted and that susceptibility to this is quite general among certain troops. Pneumonia and meningitis, especially the latter, are less readily spread by contact and susceptibility is not at all general.

It must also be pointed out that what holds true for the enlisted men is likewise true for the officers so far as distribution of disease at the various camps is concerned. Wadsworth has the lowest measles incidence among both officers and men. Pike has the highest incidence among the men and one of the highest rates for officers. In general this holds true for the other camps. Those camps with high disease incidence among the men likewise have high disease incidence among officers.

(f) *Inadequate Hospital Care.*—An effort has been made to determine whether lack of hospital accommodations has caused an excess of disease at any one camp. It is believed that this has acted to a certain degree, there having been a few instances of contact transmission within the wards. This has been corrected by more complete isolation of cases. Some camps have suffered more in this respect than others, but this has been a relatively slight factor in the general dissemination of disease. Lack of hospital facilities would seem to a



count for the excess of disease at Bowie over Travis, both camps receiving men from the same states. Bowie was cramped for hospital room and cases could not be handled as carefully as at Travis where the accommodations were more ample. With such facilities Travis was able to send incipient respiratory infections to the hospital and thus prevent their progress into more serious illness. Bowie could not do this and had partly in consequence nearly twice as many deaths from pneumonia. This result is illustrated in Chart XV. In Chart XVI is shown the apparent universal influence which treatment of the minor diseases has on the death rate from pneumonia.

## SICKNESS ADMISSIONS AND PNEUMONIA DEATHS AT CAMPS TRAVIS AND BOWIE

6 MOS. SEPT. 29, '17 TO MAR. 29, '18

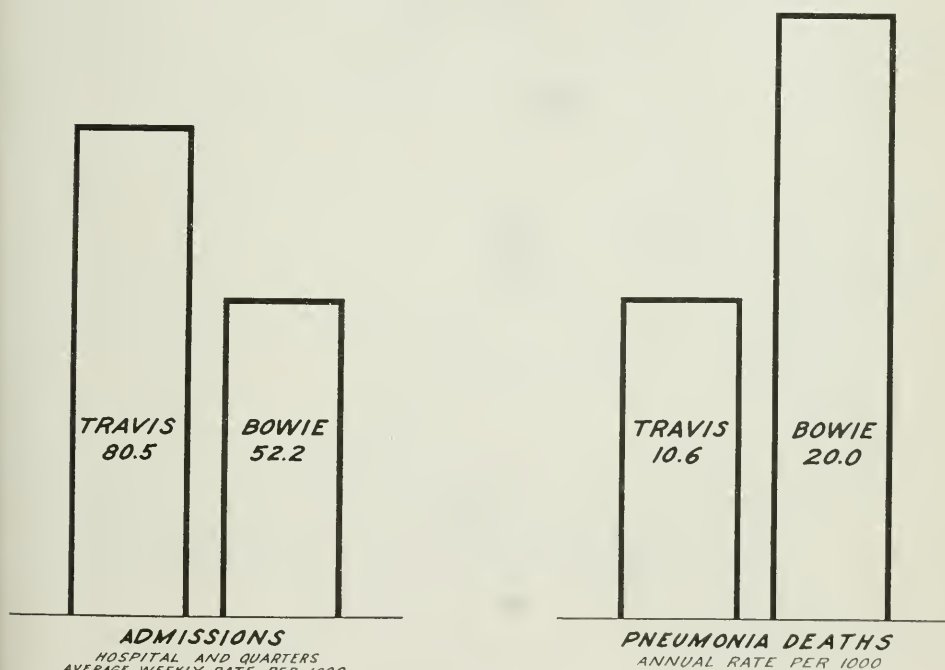


Chart XV.

Faulty quarantine at some camps is believed to have been a factor in disease transmission. Quarantine can be kept in spirit or only literally. There are a number of instances where little else was done than to issue the order. There was no following up of the order nor any serious attempt to abide by it.

(g) *Unsanitary Conditions in General.*—Respiratory disease is affected by general or personal sanitation. Inasmuch as the infective agents gain access to the body through the mouth and nose it is important to observe those matters of personal hygiene such as the avoidance of promiscuous spitting, of trading pipes and mess utensils, washing the hands before meals, etc. Evidence along

these lines is not exact or complete, but the observation has been made that in certain of these camps with high disease incidence, the men are in general more careless of their personal habits. They are not as cleanly. Spitting outdoors and indoors is of the most promiscuous character. Food is less carefully prepared and its choice is a matter of less concern than in other camps. Promiscuous and well nigh universal spitting on the streets, about and in tents, on parade grounds, etc., has been reported at Bowie. Pneumococci have been found in dust.

## RELATION BETWEEN PNEUMONIA DEATHS AND TOTAL ADMISSIONS FOR SICKNESS

NATIONAL GUARD AND NATIONAL ARMY CAMPS  
6 MOS. SEPT. 29, 1917 TO MAR. 29, 1918

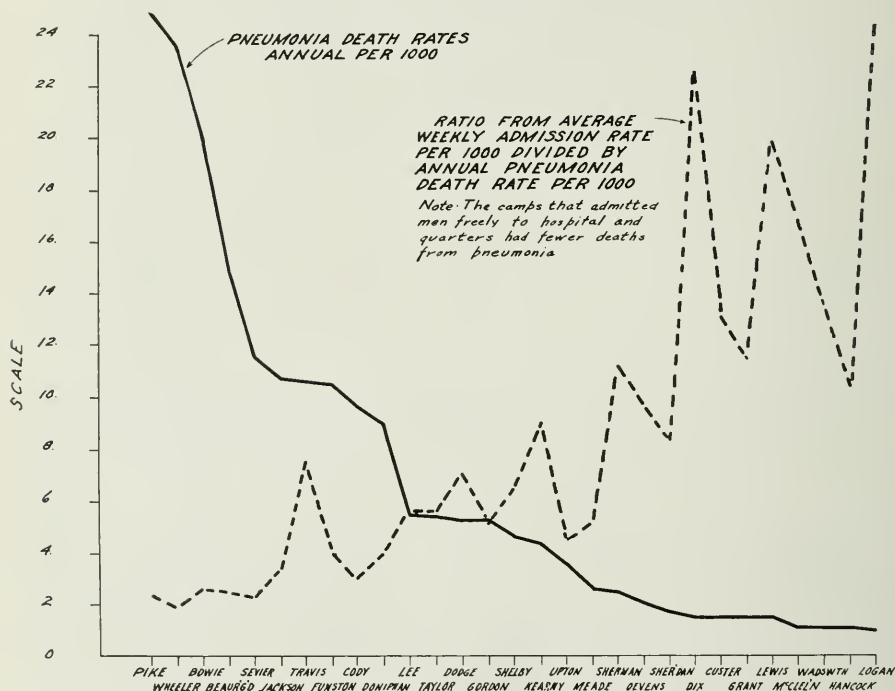


Chart XVI.

### 3. NATURAL SUSCEPTIBILITY TO DISEASE

Having considered the effect of camp life in weakening the physical tone of the soldier and the extent to which infectious disease has been spread, we come to a consideration of the innate susceptibility of the soldier or his natural immunity to disease.

If this factor is a predominating one we should expect those men who are exposed to the same general climatic influences to show similar characteristics. Or we should expect like tendencies to be exhibited by men from the same section of the country. Still further we might look for differences between city men and country men.

(a) *Racial Influence.*—The high incidence of disease at southern camps such

as Pike, Wheeler, Beauregard, Jackson, Bowie and Travis suggests that the number of negro troops there may have something to do with it, for statistics in civil life show the negro to be much more susceptible to such diseases as tuberculosis and pneumonia.

Reports from Wheeler and Beauregard show that they had no negro troops during the six months covered by this study. At Travis the proportion of white to colored troops was seven to one and the sick rates were no greater in the latter than among the former. About 25 per cent of the troops at Pike were colored. The sick rates among colored troops here was greater than among whites. We have no data as to the proportion of colored troops at Jackson, but we believe it was very small indeed if in fact there were any. With northern bred negroes at Logan there was but little difference in the sick rate between the races. With southern bred negroes in northern camps both the morbidity and mortality rates were much higher among the negroes.

There is no ground for attributing the excessive sick rates at the southern camps to colored troops.

(b) *Effect of Urban Life.*—It is a well recognized fact that respiratory disease is more common in centers of population where its means of transmission is so much readier than among the rural population. The table below well illustrates this fact and these data are typical.

TABLE 27  
URBAN AND RURAL DEATH RATES, ANNUAL RATE PER 100,000  
1915

	MEASLES	SCARLET FEVER	DIPHTHERIA AND CROUP	PNEUMONIA	TUBERCULOSIS OF THE LUNGS
Cities in Registration States	7.1	4.0	17.5	156	134
Rural part of Registration States	3.4	3.1	12.9	106	112

Analyzing the situation in the army with this question of population in mind we are brought face to face with some very interesting facts. These are illustrated in Chart XVII. Here we have compared the death rates among the soldiers from different sections of the country with the percentage rural population of that section. The association of high disease incidence and rural population is very suggestive.

The death rates are distributed roughly in three groups. Those representing the West South Central section, the East South Central Section, and the South Atlantic stand first with the highest rates. Next with a rate just half that of the former group comes the West North Central. Next come the other sections, the difference between them being slight. New England is the least rural and has next to the lowest death rate. New England is represented by Camp Devens which contains draft men. If New England were represented in this table by a larger proportion of city men as are the other sections with their National Guard units, it is very possible that New England's death rate would be lower than it is.

The similarity in these two curves is believed to be of the greatest sig-

nificance. It will be noticed that they are not parallel throughout. There are several explanations for this. In the first place, the exactness of the two sets of data is not sufficient for a complete correlation, secondly it is felt that the disparity in the curves carries some significance. It means that factors other than sparseness of population are at work in the first two sections causing them to run unduly high. The first two sections which are more than twice the rate of the third are the only ones including southern territory. There is thus brought to light a secondary broad general influence which distinguishes different sections of the country.—This is climate. It seems reasonable to explain the high position of the first two points then as due (1) to rural population and (2) to climate. This latter influence we shall discuss in a later paragraph.

The influence of rural life on disease incidence as brought out in this table

RELATION BETWEEN RURAL LIFE AND DEATH RATES  
AT NATIONAL GUARD AND NATIONAL ARMY CAMPS

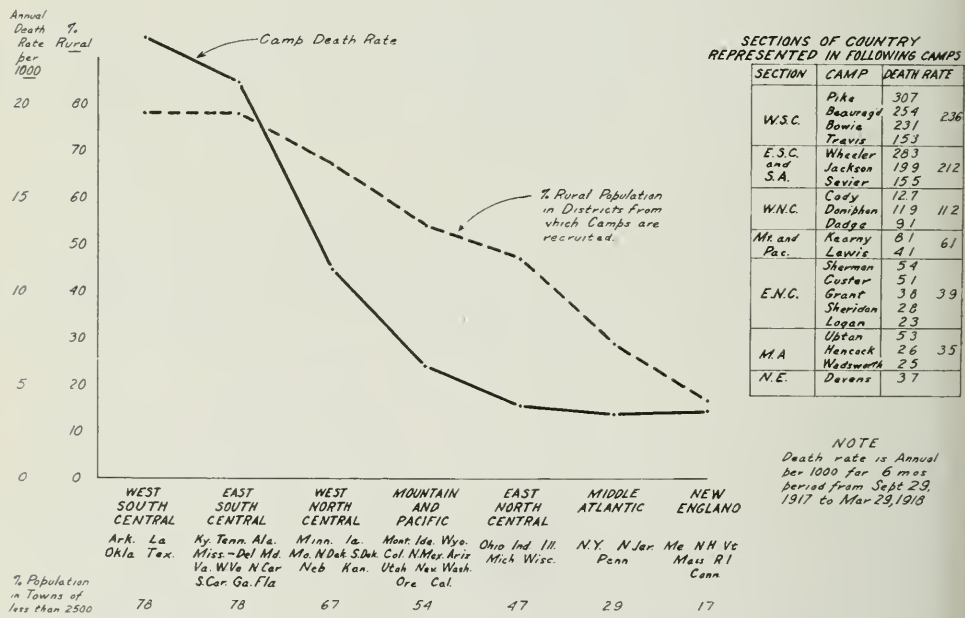


Chart XVII.

is further supported by the figures gathered from the city and rural units in the camp. Camp Cody reports that disease incidence was 48 per cent higher in the 134th Infantry than in the 133rd. The latter is made up of troops from the larger cities of Iowa. The former included troops mainly from the smaller towns of Nebraska.

Similarly disease incidence was 51 per cent greater in the 136th Infantry made up from the smaller towns of Minnesota than in the 135th Infantry made up of men from the larger cities of this state.

The excess among rural troops of such diseases as measles, mumps and scarlet fever has been observed at Camp Custer and at Camp Wheeler.

Camp Wadsworth reports that their division made up of Guardsmen from the larger cities of New York State was practically free from disease until



March when about 1500 draft men from the mountains of Tennessee and Kentucky were received. Their arrival had a marked effect upon the disease rate. These men soon developed meningitis, pneumonia and the minor communicable diseases. Their noneffective rate was three times that of the original division.

The epidemiologist at Camp Doniphan points out the unusually low disease incidence among city troops as compared with those from the country. The 138th Infantry and 128th Machine Gun Battalion were recruited from St. Louis, Missouri. Their annual pneumonia morbidity rates from October to March were 15. and 25. respectively. The 137th Infantry and 129th Field Artillery were from the small towns of Kansas. Their corresponding rates were 65. and 50. respectively.

(c) *Climatic Influence*.—A climatic influence on disease incidence independent of the rural influence has already been mentioned. The relative weights of each is difficult to measure, but that they have an independent existence is quite evident. The most striking illustration of this is offered by the two maps on which are located the death rates from all causes for each camp, Charts XVIII and XIX. In the first map the circles are located at the site of the camp. It will be observed that the south is covered by circles of all shades. Logan in Texas has a death rate less than 8.0 per 1000. Travis has a rate somewhat higher. Funston represents the third group of death rates ranging from 16.0 to 24.0. The highest rate shown by the black circle is represented by Pike and Beauregard. All five of these camps are subject to approximately the same climatic influence and yet their death rates are strikingly different. Looking now at the second map we see an entirely different story. In this case the circles are located in the center of the region from which the troops are recruited. The clear circles, with a single exception, have now disappeared from the south and we find all but two located in the northern states east of the Mississippi, and this section contains none but clear circles.

The exceptions are Lewis in the northwest and Gordon in the south. Gordon would seem to controvert the evidence we have presented on this score and a great deal of speculation was raised as to why Gordon alone should exhibit characteristics different from that shown by all other southern troops. Some time elapsed before an explanation was forthcoming. Gordon, it seems, was originally set apart for troops from Georgia, Alabama, and Tennessee. About six weeks after the camp opened the above named troops were transferred to Camp Wheeler and their place was filled by men from 21 different states, largely drawn from the north. Gordon, thus, in having a death rate below 8.0 is simply exhibiting a characteristic common to the northeastern section of the country.

Evidence showing the greater susceptibility of southern troops and certain lights on the causes is to be had from the reports of the medical officers attached to these camps.

*Camp Sevier*.—"From 25 to 33 per cent of all cases submitted to the Base Hospital came from the mountains of East Tennessee and the Carolinas. Almost all cases of bronchopneumonia were from men of this locality. A large percentage of the men from South Carolina were suffering from hookworm infection. The percentage runs as high as 35%."

"Tennessee, North and South Carolina have furnished the largest proportion of the troops. The troops from other states have not furnished as large a percentage of sick as the troops from the above-mentioned states, but have been the means of introducing or re-introducing some of the contagious diseases."

*Camp Wadsworth*—The greatest prevalence of sickness occurred among troops from

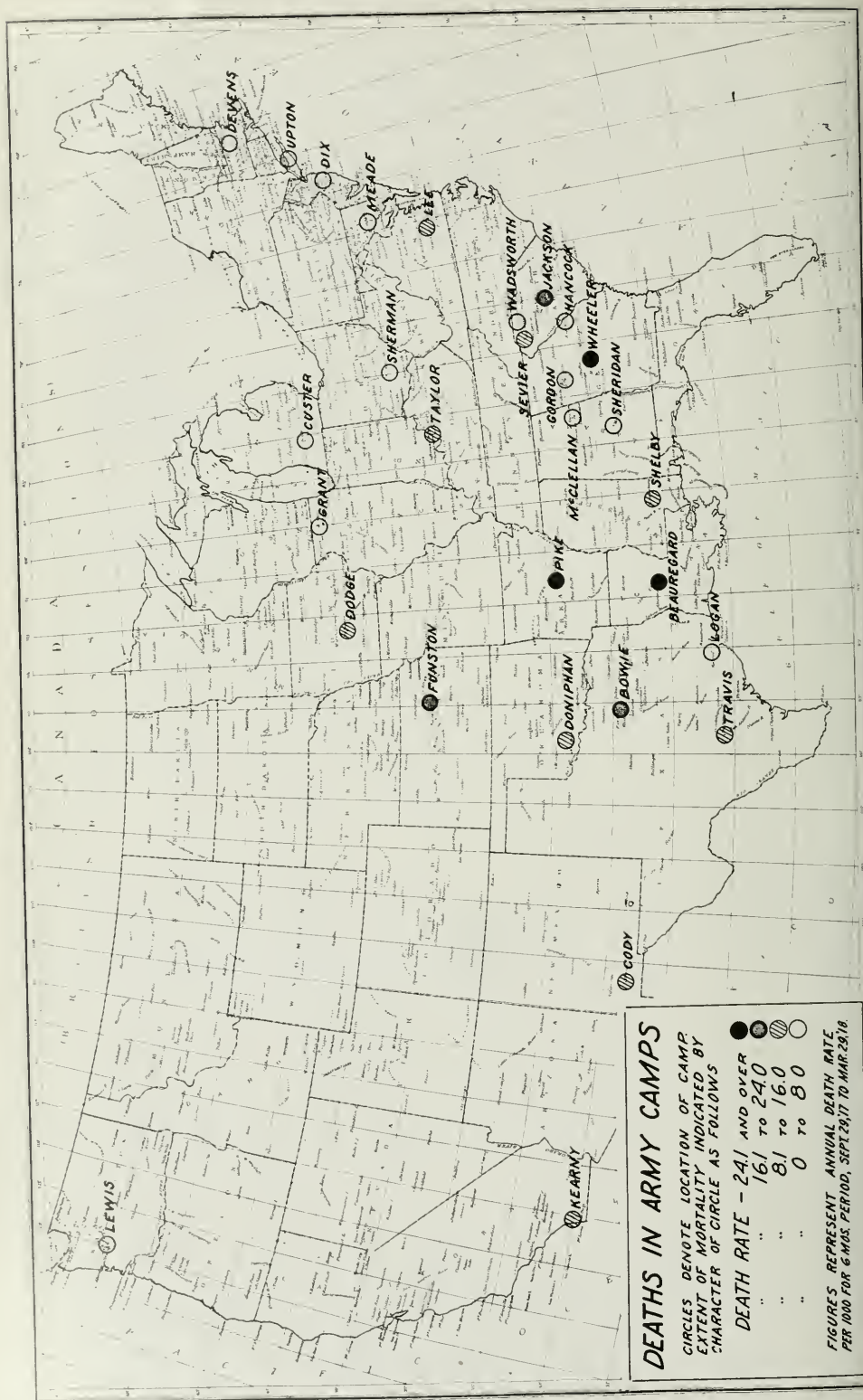


Chart XVIII.

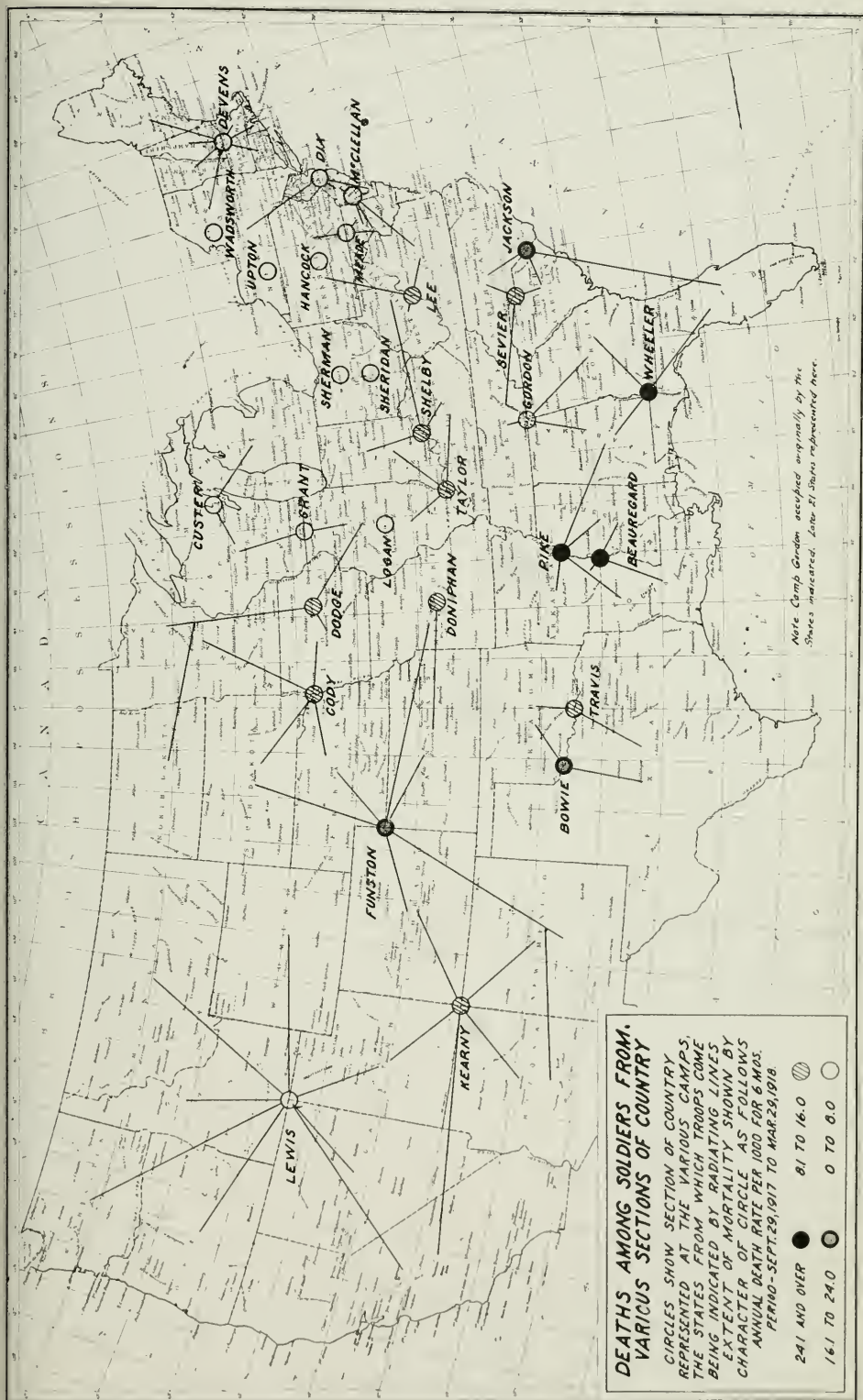


Chart XIX.



Kentucky, who "brought with them measles, mumps, meningitis, pneumonia and hookworm. These were troops of low resisting powers from the mountains of Kentucky."

*Camp Wheeler*.—The men at Camp Wheeler were recruited mainly from Alabama, Georgia and Florida. That the location and previous environment of the troops here were responsible for the epidemic of measles and pneumonia is attested by the report of the Division Surgeon. He speaks as follows about the physical condition of the men:

"1. A class of drafted men of most miserable physique is noticed by all observers.

2. The men from the Gulf Coast could not withstand even the mild winter of Central Georgia.

3. Many of the men suffered from the anemia of hookworm or malaria."

*Camp Pike*.—As to the fundamental causes underlying the epidemic at this camp, the following may be said:

1. "Low resistance on the part of draft troops coming from the States of Mississippi, Louisiana, Arkansas and Alabama. A study of the number of men from these sections of the country turned down by our examining board demonstrates beyond question that in the majority of instances we are dealing with a very inferior class of draft men. Company Commanders have, for example, told me of the difficulty of getting their men to eat the ordinary army food. Many of them have lived for years on corn bread and side meat."

*Camp Jackson*.—"The enlisted personnel of this camp seemed to show an unusual amount of illiteracy, general ignorance and ignorance of personal hygiene, all of which doubtless aided in the propagation of contagious disease. An illuminating example of the state of sophistication is the fact that classes were formed and instruction given in the use of water closets and toilet paper. The enlisted personnel comes mainly from North and South Carolina and Florida. It is mainly rural in origin and contains a relatively large proportion of men who have not had measles or mumps in childhood."

"The physical condition of the men is not more than average, and I feel sure that there must be something in the old theory of lowered resistance and other conditions having an influence on infection. About 30 per cent of the men are found to have hookworm, while about 3 per cent are suspected of having malaria."

This evidence seems sufficient to establish the physical inferiority of the men from this region. Turning again to our morbidity and mortality statistics we find still further verification of this fact. Where there is a specific susceptibility to any disease as may readily occur in persons of apparently good physical tone, we need not be surprised at the outbreak of an epidemic of that disease but we do not look for high incidence in other diseases as well. The troops from the Southern States possess a susceptibility that is general as well as specific. They are subject not only to the ravages of pneumonia, but also to other diseases as well. Their death rate from all causes is higher and their sickness incidence is greater. This fact has already been brought out by Chart III early in this report. Here eleven indices of physical well-being have been plotted for each of the 29 National Guard and National Army camps. The camps have been graded according to their relative standing in each index and the position of the squares have been placed in order of the death rate from all causes. The significant features of this Chart is that the camps with Southern troops top the list. The blackness of the square shows that these camps are not only high in one cause of sickness, but are universally high in everything. Pike, for instance, has the highest (1) death rate from all causes, (2) death rate from diphtheria, (3) morbidity from measles, (4) morbidity from scarlet fever, (5) morbidity due to venereal disease.

Wheeler is highest in deaths from tuberculosis and a close second in pneumonia morbidity.

Beauregard has the highest malaria morbidity.

Bowie stands first in pneumonia morbidity; Jackson first in meningitis morbidity, Travis first in hospital admission rate.

Five camps, Pike, Wheeler, Beauregard, Bowie and Jackson, have among



them nine out of eleven first places in the various causes which make soldiers noneffective.

Another fact which has a direct bearing on this question is that not only is the general sickness greater among Southern troops, but once sick their chances of recovery are less. Thus in Table 28 are given the morbidity and mortality rates for pneumonia and meningitis for certain camps. The Southern troops are seen to average a greater case mortality in both diseases. The Pike group have a pneumonia case mortality of 28 per cent, and a meningitis case mortality of 61 per cent. These figures are nearly twice those of the Upton group with 16 per cent and 37 per cent respectively.

TABLE 28  
CASE MORTALITY FROM PNEUMONIA AND MENINGITIS IN CERTAIN CAMPS

CAMP	PNEUMONIA			MENINGITIS		
	MORBIDITY RATE	MORTALITY RATE	CASE MORTALITY %	MORBIDITY RATE	MORTALITY RATE	CASE MORTALITY %
Pike	63.	25.	40	3.8	2.4	63
Bowie	96.	20.	21	4.7	1.6	54
Beauregard	42.	15.	36	12.8	7.7	60
Wheeler	95.	24.	25	2.4	2.1	88
Jackson	36.	11.	31	25.7	7.5	29
Travis	78.	11.	14	2.7	2.5	93
Average			28			61
Upton	15.	3.6	24	.54	.34	63
Wadsworth	8.8	1.1	13	.78	.20	26
Dix	8.0	1.5	19	.57	.19	33
Logan	16.	1.0	6	.73	.22	30
Hancock	6.7	1.1	16	1.8	.44	24
Devens	9.8	2.0	20	.63	.28	44
Average			16			37

#### SUMMARY OF THE CAUSES OF RESPIRATORY DISEASES IN ARMY CAMPS

From this extended review of the situation we feel that the greatest single factor in the prevalence of disease in certain camps and their absence in others has been the natural susceptibility of the men. Disease incidence has been markedly greater among Southern troops. Southern troops are as a class more susceptible to respiratory disease. They are more susceptible because they have not had these diseases in childhood and therefore have established no immunity to them. They are more susceptible also because they have weaker physiques to begin with. This physical inferiority is due to ignorance of and consequent failure to act upon the fundamental laws of sanitation. In consequence those debilitating diseases such as hookworm, malaria and pellagra prevail. Venereal disease is excessive. Insufficient attention is paid to the character and quality of food consumed; these men have not learned that diseases are transmitted by germs most of which gain access to the body through the mouth and nose. Spitting is promiscuous. There is no thought of stopping it even in tents and barracks. The reason for stopping it is not even appreciated.

With this condition of affairs to start with we find that there have been added those aggravating factors such as exposure, fatigue, lack of warm clothing, cold quarters by day, cold quarters and insufficient bedding by night. These

men were naturally susceptible. Lessen their resistance still further, introduce the carrier case and it is not at all difficult to anticipate the result. The fire rages among this highly inflammable timber.

The value and purpose of an epidemiological study is to discover facts that will prevent a recurrence of the trouble. Now that we have established the case how are we to act upon it?

First and foremost it seems necessary to graduate the introduction of civilians into army life. The change has been too abrupt. Men should be called first to a semiactive reserve army. Here they should get drill and the essentials of sanitation and self care by lecture and by demonstration. The drill and calisthenics should be the hardening process. After this the transfer should be made to camp where a man's entire time is given over to his military training.

Before entering camp men should be examined for incipient disease. The suspects should be separated and watched before their dispatch to camp. Vaccination for typhoid and smallpox can be completed while in the reserve force.

Once established in camp the transfer of men from one camp to another should not take place without a careful examination and removal of those who show signs of illness. This will prevent this all too frequent transportation of sick men, who are dangerous to others because of their sickness.

These precautions together with care in the proper mixture of work and rest, judicious selection in the quality and balancing of the food ration, the adequate protection of the man, especially the one from the warm climate, against cold and exposure, his protection against the sick through effective quarantine measures, and discretion in the use of the physical hardening process should moderate to a large degree the experiences of the past winter.

## RESPIRATORY DISEASE AT ARMY CAMPS

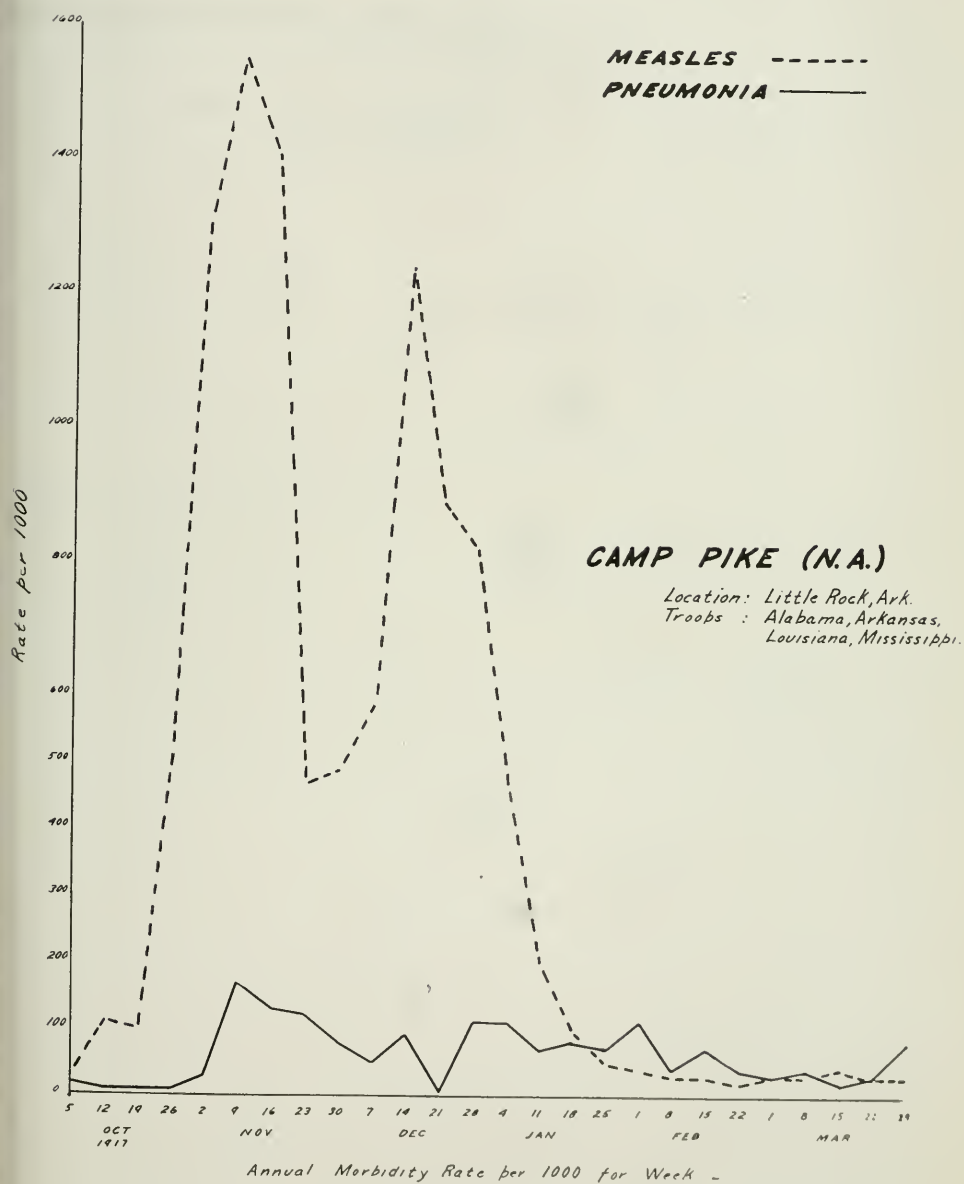


Chart XX.

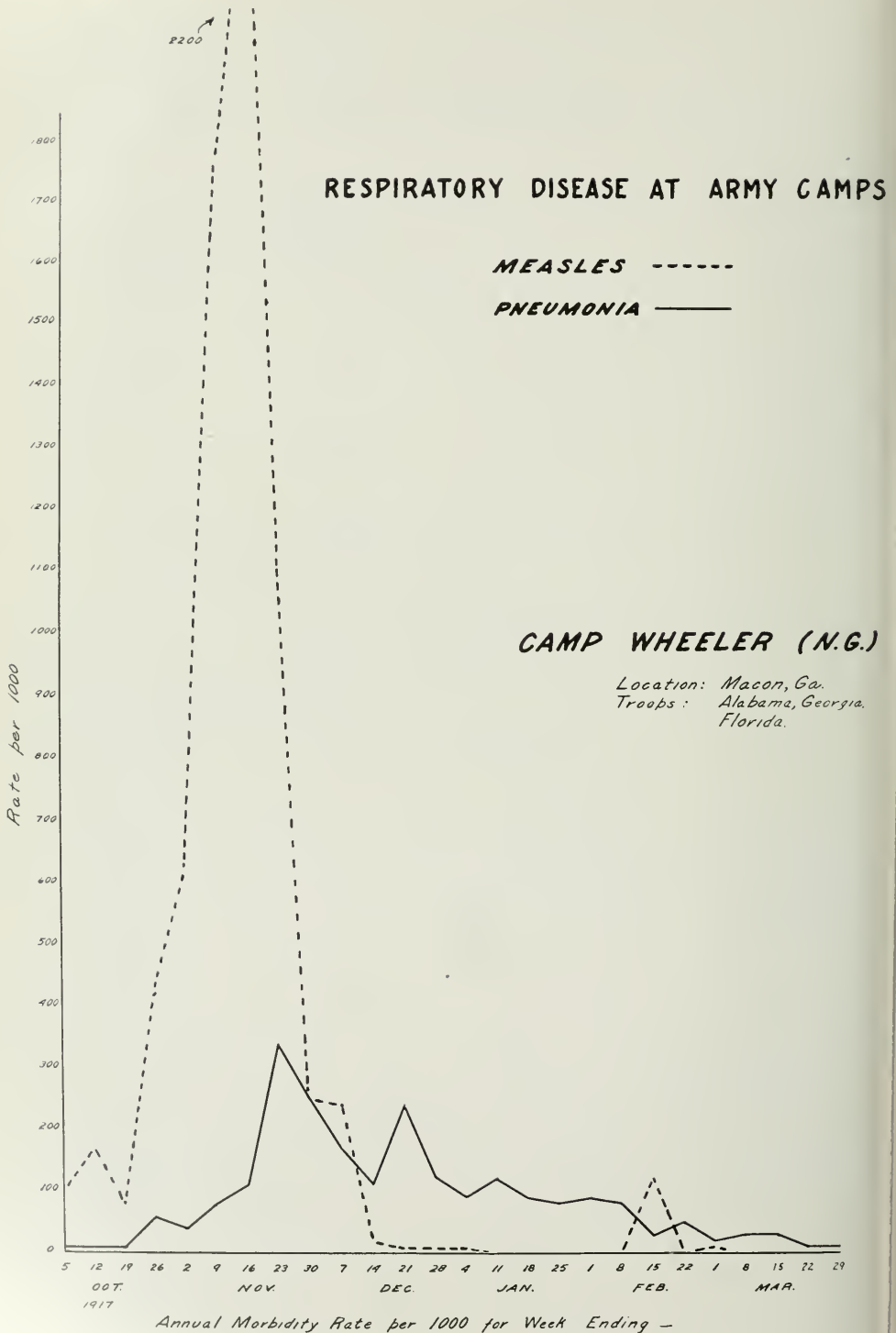


Chart XXI.



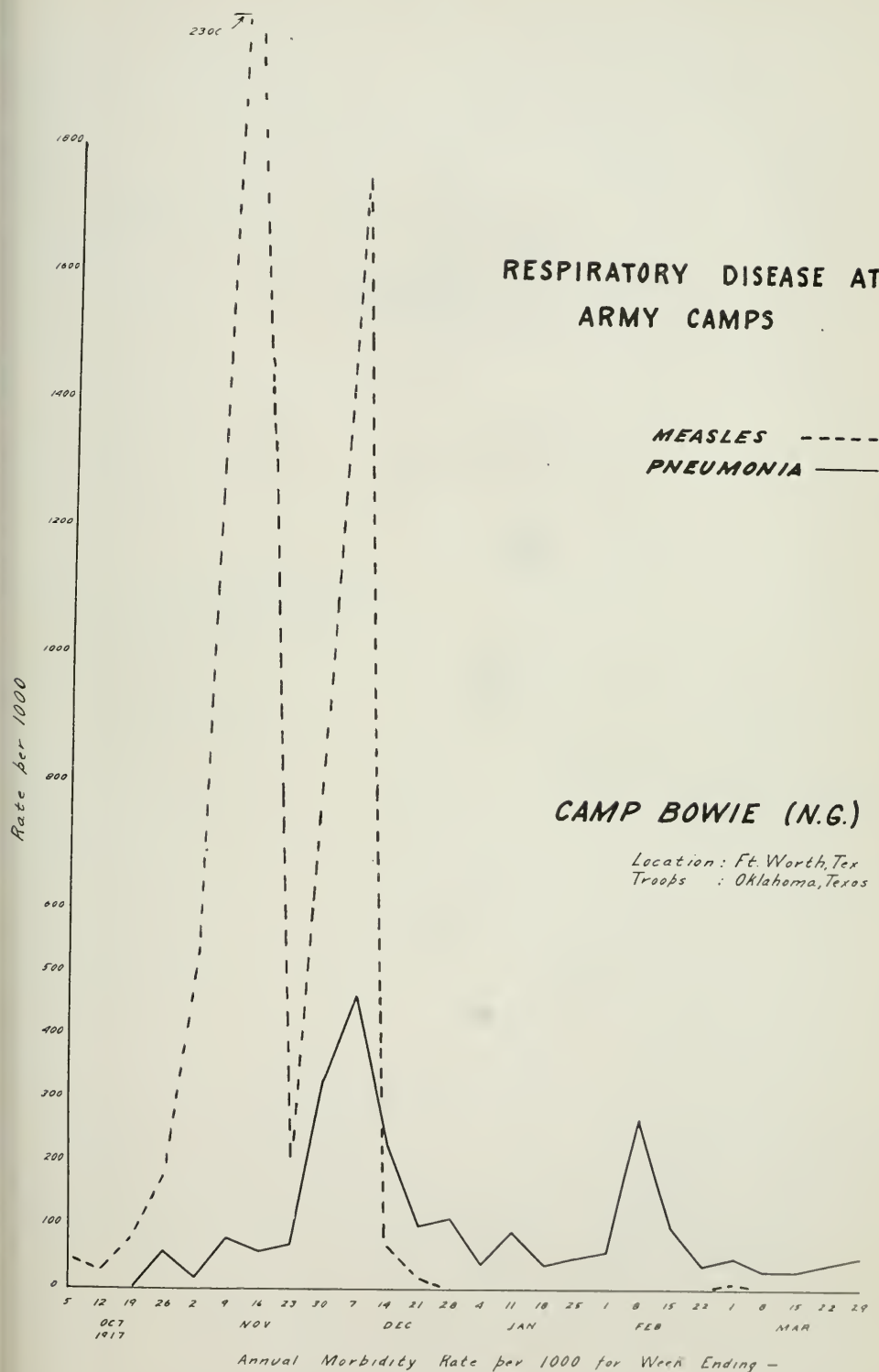
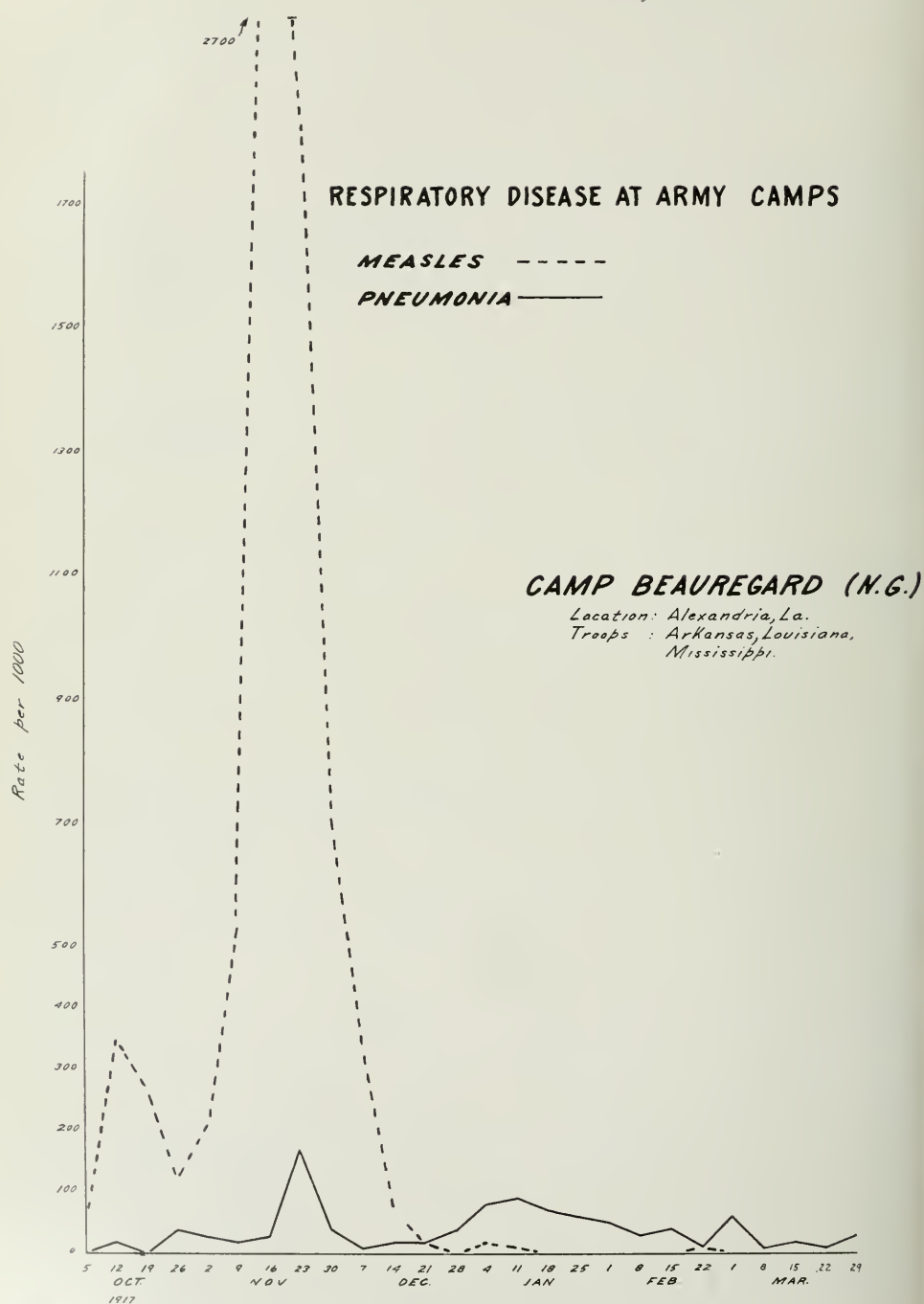


Chart XXII.



Annual Morbidity Rate per 1000 for Week Ending —

Chart XXIII.

## RESPIRATORY DISEASE AT ARMY CAMPS

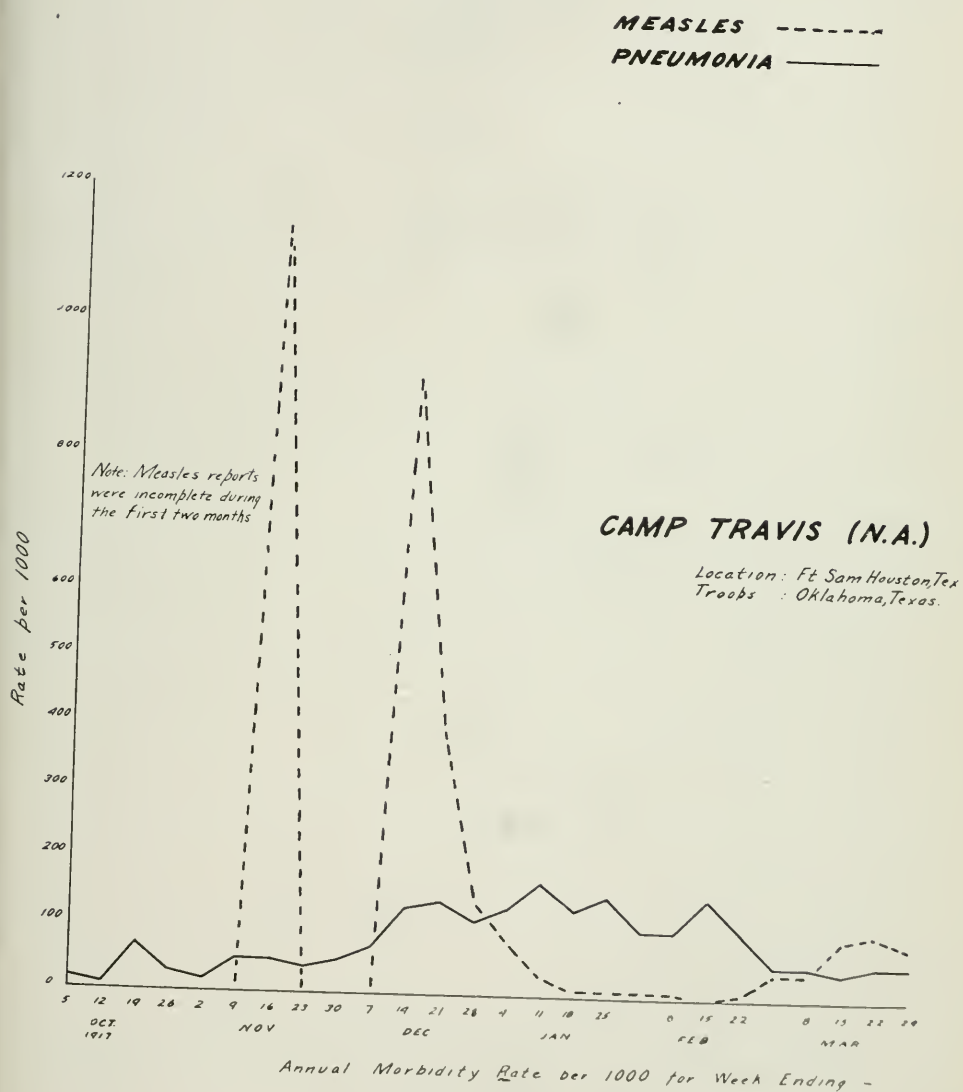


Chart XXIV.

## RESPIRATORY DISEASE AT ARMY CAMPS

MEASLES - - - - -  
PNEUMONIA ———

## CAMP SEVIER (N.G.)

Location : Greenville, S.C.  
Troops : Tennessee  
N. Carolina,  
S Carolina

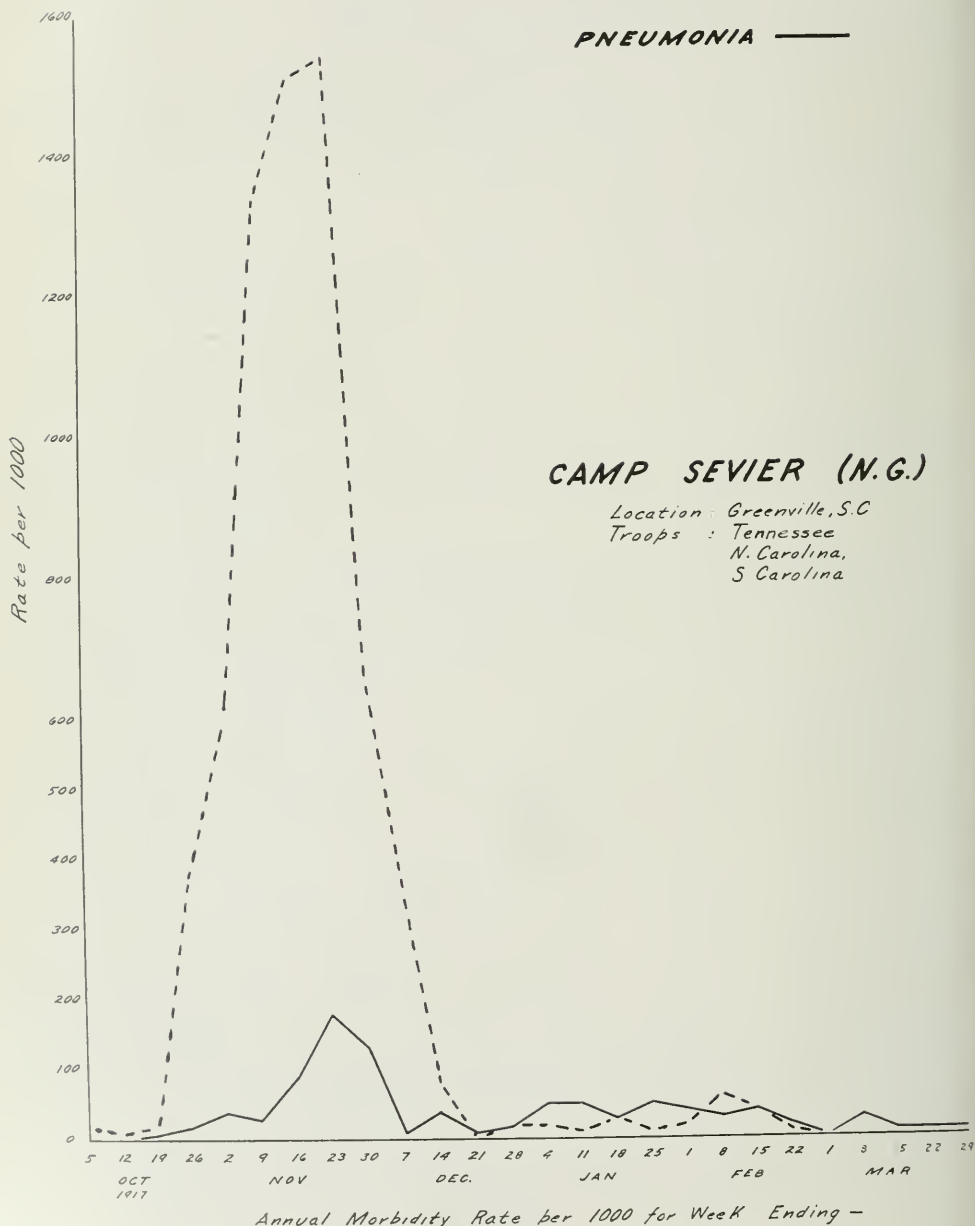


Chart XXV.



## RESPIRATORY DISEASE AT ARMY CAMPS

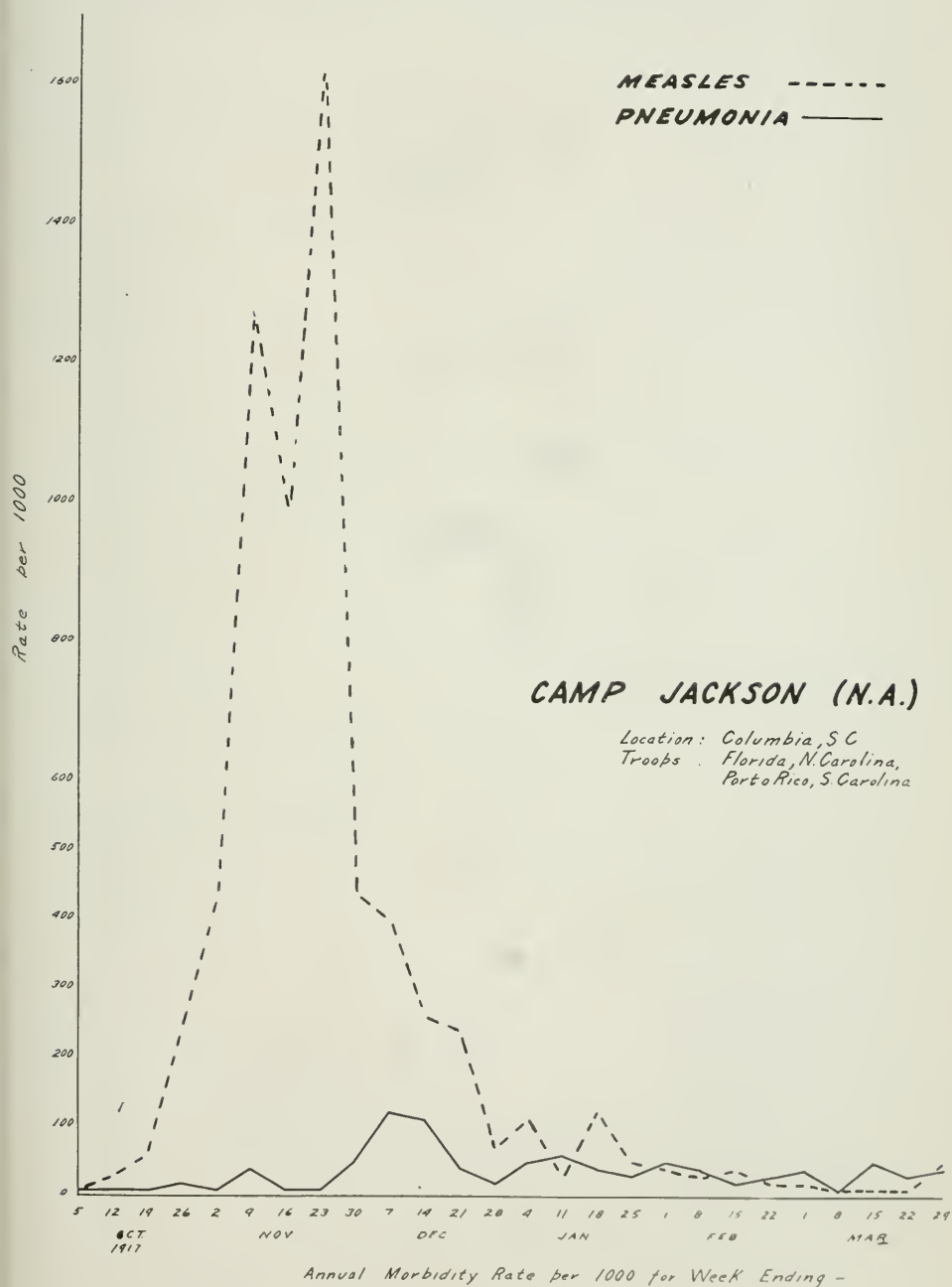


Chart XXVI.

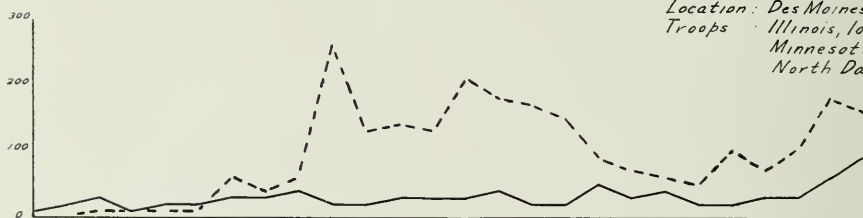
## RESPIRATORY DISEASE AT ARMY CAMPS

MEASLES - - - - -

PNEUMONIA ———

## CAMP DODGE (N.A.)

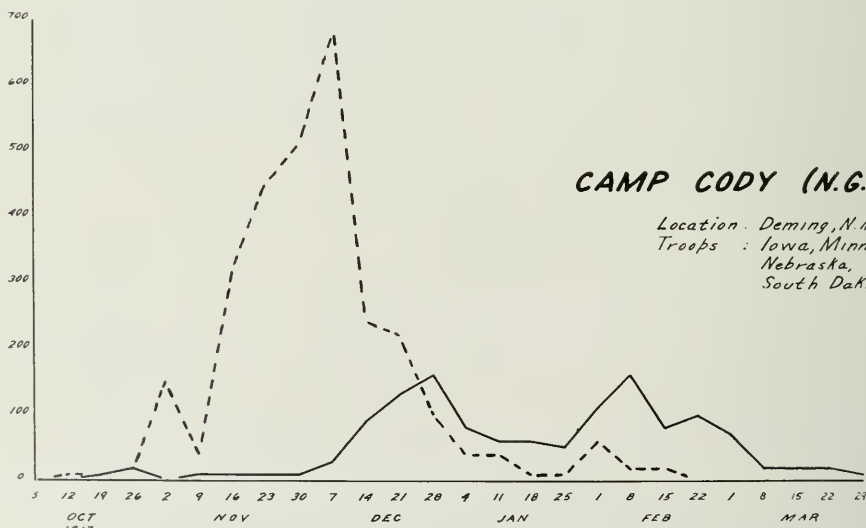
Location: Des Moines, Ia.  
Troops: Illinois, Iowa,  
Minnesota,  
North Dakota



Rate per 1000

## CAMP CODY (N.G.)

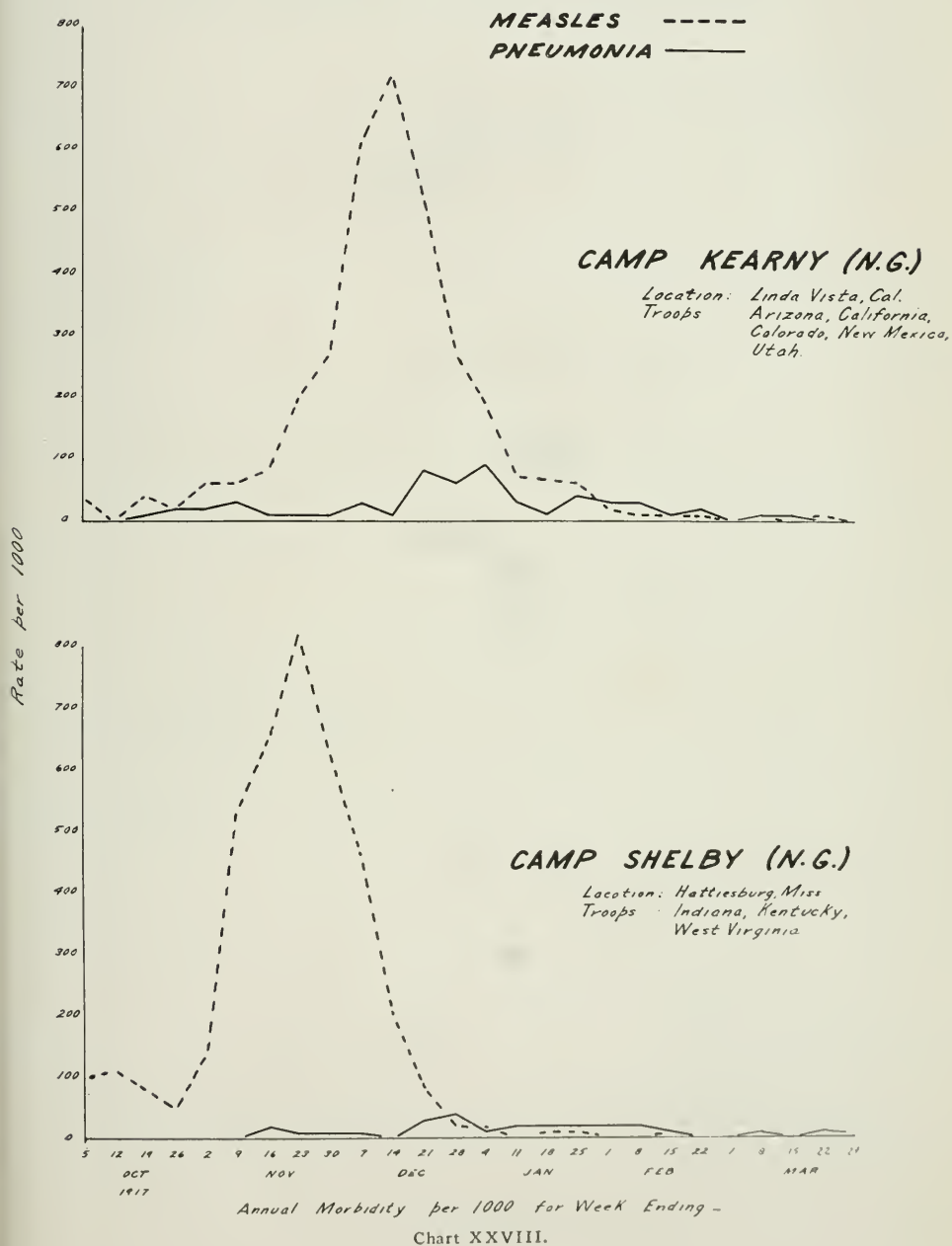
Location: Deming, N.M.  
Troops: Iowa, Minnesota,  
Nebraska,  
South Dakota



Annual Morbidity Rate per 1000 for Week Ending —

Chart XXVII.

## RESPIRATORY DISEASE AT ARMY CAMPS



## RESPIRATORY DISEASE AT ARMY CAMPS

MEASLES -----  
PNEUMONIA ————

## CAMP LEWIS (N.A.)

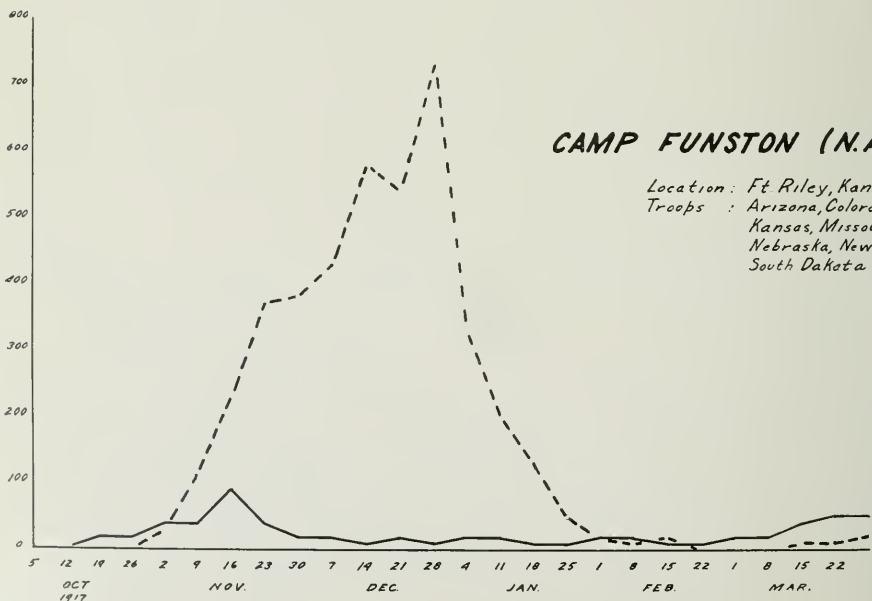
Location: American Lake, Wash.  
Troops: Alaska, California,  
Idaho, Montana, Nevada,  
Oregon, Utah,  
Washington, Wyoming



Rate per 1000

## CAMP FUNSTON (N.A.)

Location: Ft. Riley, Kan.  
Troops: Arizona, Colorado,  
Kansas, Missouri,  
Nebraska, New Mexico,  
South Dakota



Annual Morbidity Rate per 1000 for Week Ending -

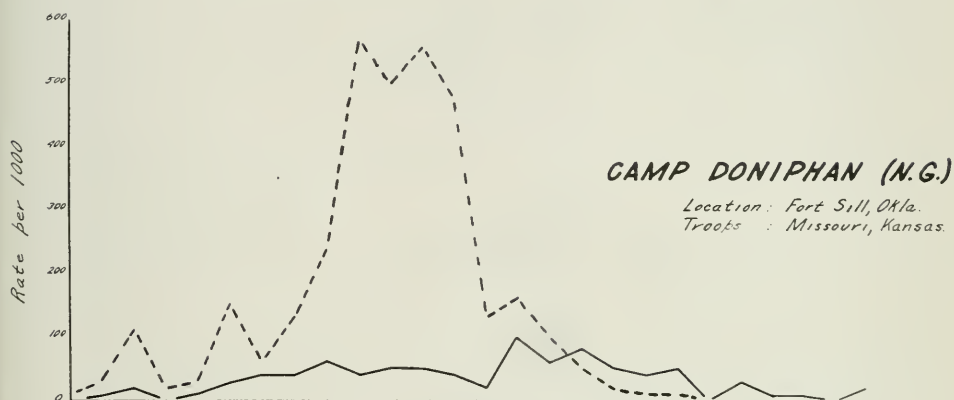
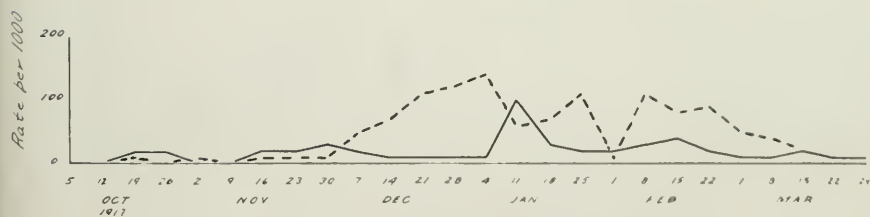
Chart XXIX.



## RESPIRATORY DISEASE AT ARMY CAMPS

MEASLES - - - - -

PNEUMONIA ———

**CAMP LOGAN (N.G.)**Location: Houston, Tex.  
Troops: Illinois

Annual Morbidity Rate per 1000 for Week Ending -

Chart XXX.

## RESPIRATORY DISEASE AT ARMY CAMPS

MEASLES -----

PNEUMONIA —————

## CAMP McCLELLAN (N.G.)

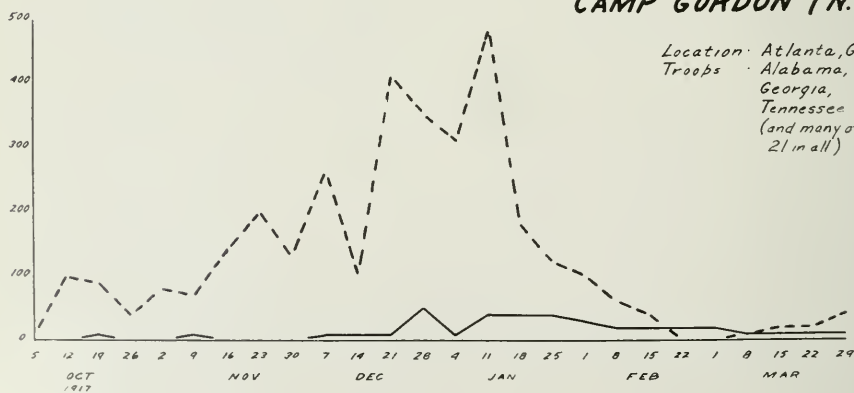
Location Anniston, Ala.  
Troops Delaware, Dist. Columbia,  
Maryland, New Jersey,  
Virginia



Rate per 1000

## CAMP GORDON (N.A.)

Location Atlanta, Ga.  
Troops Alabama,  
Georgia,  
Tennessee  
(and many other States  
21 in all)



Annual Morbidity Rate per 1000 for Week Ending -

Chart XXXI.

## RESPIRATORY DISEASE AT ARMY CAMPS

MEASLES -----

PNEUMONIA —————

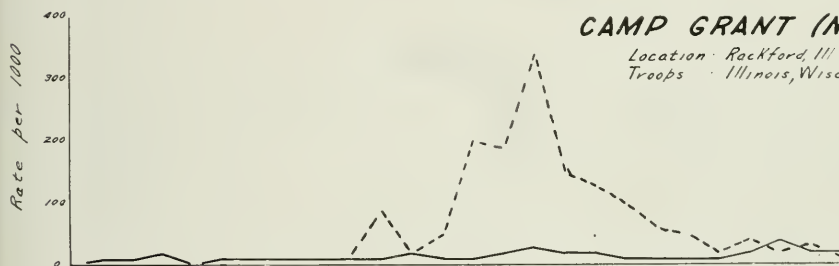
## CAMP CUSTER (N.A.)

Location Battle Creek, Mich.  
 Troops Michigan, Wisconsin



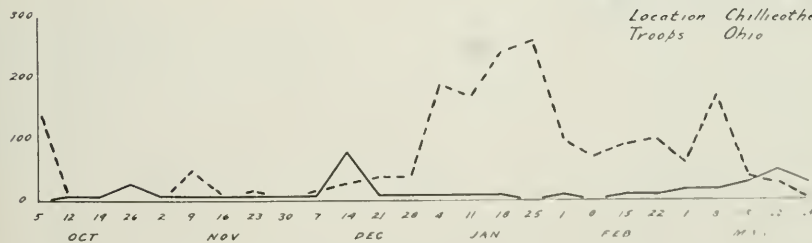
## CAMP GRANT (N.A.)

Location Rockford, Ill.  
 Troops Illinois, Wisconsin



## CAMP SHERMAN (N.A.)

Location Chillicothe, Ohio  
 Troops Ohio

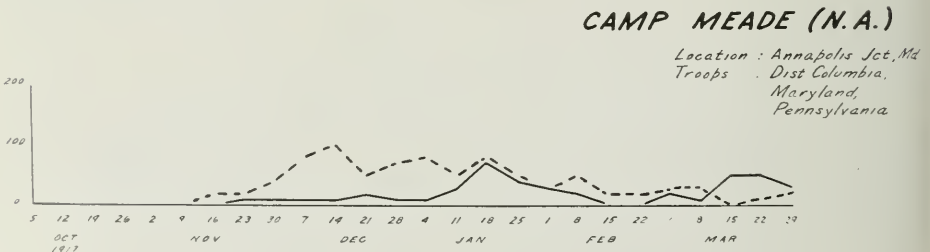
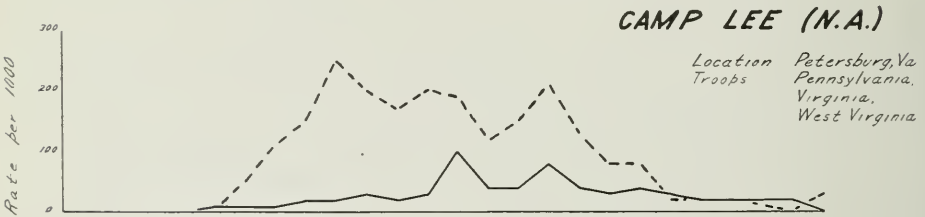
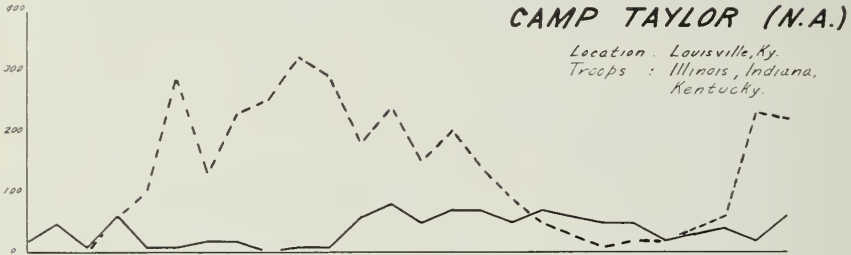


Annual Morbidity per 1000 for Week Ending —

Chart XXXII.

# RESPIRATORY DISEASE AT ARMY CAMPS

MEASLES -----  
PNEUMONIA ———



Annual Morbidity Rate per 1000 for Week Ending -

Chart XXXIII.



## RESPIRATORY DISEASE AT ARMY CAMPS

MEASLES -----

PNEUMONIA —————

## CAMP DIX (N.A.)

Location Wrightstown, N.J.  
Troops Delaware New Jersey,  
New York



## CAMP UPTON (N.A.)

Location Yaphank, L.I.  
Troops New York



## CAMP DEVENS (N.A.)

Location Ayer, Mass  
Troops Connecticut, Maine  
Massachusetts,  
New Hampshire,  
Rhode Island,  
Vermont, New York



Annual Morbidity per 1000 for Week Ending

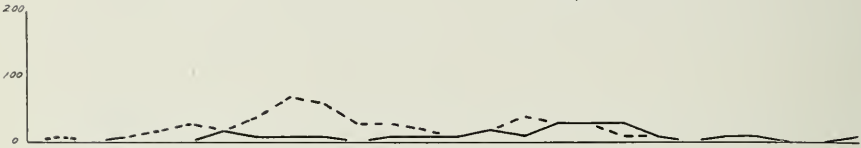
Chart XXXIV.

RESPIRATORY DISEASE AT ARMY CAMPS

MEASLES      - - - -  
PNEUMONIA    - - - -

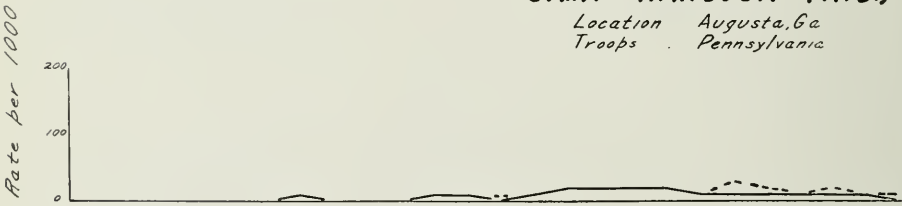
CAMP SHERIDAN (N.G.)

Location    Montgomery, Ala  
Troops      : Ohio



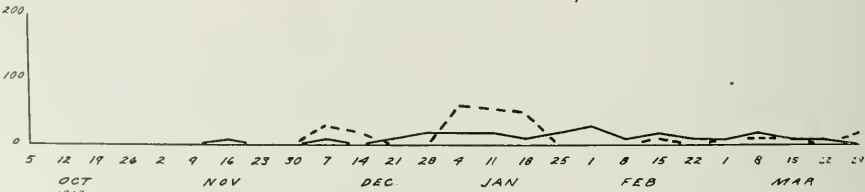
CAMP HANCOCK (N.G.)

Location    Augusta, Ga  
Troops      : Pennsylvania



CAMP WADSWORTH (N.G.)

Location    Spartanburg, S.C  
Troops      : New York



Annual Morbidity Rate per 1000 for Week Ending —

Chart XXXV.

## TABLE OF CONTENTS

**The Communicable Diseases in the National Guard and National Army of the United States during the Six Months from September 28, 1917, to March 29, 1918.**

	Page
Preface .....	635
Introduction .....	635
Comparative Mortality in Army and Civil Life.....	636
Comparative Death Rates in National Guard and National Army.....	664
Causes of Death in the Army.....	645
Sickness in the Army.....	648
Epidemic Disease in National Guard and National Army Camps.....	649
Pneumonia .....	649
Differences in the Virulence of the Infecting Organisms Causing Pneumonia....	653
Difference in Susceptibility to Pneumonia of the Men in Different Camps and the Experiences of the Civil War in this Connection.....	655
Measures for Protection against Pneumonia and Other Respiratory Disease.....	660
Measles .....	662
Meningitis .....	666
Scarlet Fever .....	668
Differences in the Behavior of Measles, Pneumonia, Meningitis and Scarlet Fever	670
Typhoid and Paratyphoid .....	672
Diphtheria .....	674
Tuberculosis .....	674
Epidemic Bronchitis .....	675
Influenza .....	676
Analysis of Causes of Disease in the Army.....	679
1. Influence of these Factors which bring on Physical Debility.....	681
a. Exposure to severe weather.....	681
b. Insufficient clothing .....	683
c. Inadequate housing—lack of heat.....	684
d. Fatigue .....	684
2. Unusual Facilities for the Transmission of the Infective Agent.....	685
a. Close Contact with Carrier Cases.....	685
b. Undetected Cases among New Recruits.....	687
c. Importation of Mildly Sick Men from Other Camps.....	687
d. Association with Civilian Community.....	688
e. Overcrowded Quarters .....	689
f. Inadequate Hospital Care of Patients.....	690
g. Unsanitary Conditions in General.....	691
3. Natural Susceptibility to Disease .....	692
a. Racial Influence .....	692
b. Effect of Urban Life .....	693
c. Climatic Influence .....	695
Summary of the Causes of Respiratory Diseases in Army Camps.....	699

## LIST OF CHARTS

Chart No.	Page
I. Death Rates from all Causes in National Guard and National Army Camps..	638
II. Comparative Mortality in Certain Army Camps and American Cities.....	640
III. Comparative Mortality and Morbidity from Various Causes in National Guard and National Army Camps.....	642

IV. Respiratory Disease Incidence in Civilian and Army Life.....	644
V. Distribution of Deaths in the Army by Cause.....	646
VI. Relative Frequency of the Various Causes of Admission to Hospital and Quarters .....	648
VII. Pneumonia in National Guard and National Army Camps.....	651
VIII. The Fruits of Preventive Medicine.....	659
IX. Measles in National Guard and National Army Camps.....	663
X. Meningitis in National Guard and National Army Camps.....	665
XI. Seasonal Occurrence of Communicable Disease in National Guard and Na- tional Army Camps .....	671
XII. Banishing Typhoid Fever from the American Army.....	673
XIII. Mean Monthly Temperature in Certain U. S. Cities.....	682
XIV. Venereal Disease in the Three Groups of the U. S. Army.....	686
XV. Pneumonia Death Rate and Sickness Admissions at Camps Bowie and Travis..	691
XVI. Relation between Pneumonia Death Rate and Total Admissions for Sickness..	692
XVII. Relation between Rural Life and Death Rates at National Guard and National Army Camps .....	694
XVIII. Map showing Death Rates in National Guard and National Army Camps....	696
XIX. Map showing Death Rates among Soldiers from Various Sections of the Country .....	697
Weekly Incidence of Pneumonia and Measles at National Guard and National Army Camps	
XX. Pike .....	701
XXI. Wheeler .....	702
XXII. Bowie .....	703
XXIII. Beauregard .....	704
XXIV. Travis .....	705
XXV. Sevier .....	706
XXVI. Jackson .....	707
XXVII. Dodge, Cody .....	708
XXVIII. Kearny, Shelby .....	709
XXIX. Lewis, Funston .....	710
XXX. Doniphan, Logan .....	711
XXXI. McClellan, Gordon .....	712
XXXII. Custer, Grant, Sherman .....	713
XXXIII. Taylor, Lee, Meade .....	714
XXXIV. Dix, Upton, Devens .....	715
XXXV. Sheridan, Hancock, Wadsworth .....	716



# The Journal of Laboratory and Clinical Medicine

Vol. III.

AUGUST, 1918

No. 11

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.  
Ann Arbor, Mich.

## ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D.	- - -	ST. LOUIS
HANS ZINSSER, M.D.	- - -	NEW YORK
PAUL G. WOOLLEY, M.D.	- - -	CINCINNATI
FREDERICK P. GAY, M.D.	- - -	BERKELEY, CAL.
J. J. R. MACLEOD, M.B.	- - -	TORONTO
ROY G. PEARCE, M.D.	- - -	CLEVELAND
ROGER S. MORRIS, M.D.	- - -	CINCINNATI
GERALD B. WEBB, M.D.	- - -	COLORADO SPRINGS
E. E. SOUTHARD, M.D.	- - -	BOSTON

Contents of this Journal Copyright, 1918, by The C. V. Mosby Company—All Rights Reserved  
Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

## EDITORIALS

### *Convulsions*

THERE are two factors which, together, cooperate in producing the symptom which we call a "convulsion." One of these we may call the chemical factor; the other the mechanical. The one, the chemical, acts in producing edema of the brain, which so long as there is a sufficient blood supply shows itself in headache, moderate dilatation of the pupils, and other changes which are suggestive of more serious symptoms which are convulsive depending upon the amount of blood that reaches the swollen central nervous system. The amount of blood reaching the brain depends upon the difference between the intravenous pressure and the intracerebral pressure. As long as the cerebral swelling is not great enough to bring the difference in intravenous and intracerebral pressure too close to zero a convulsion does not occur. Also as long as the intravenous pressure remains enough above the intracerebral pressure to adequately supply the brain with blood, the convulsion will not occur.

Ervin<sup>1</sup> has studied the relation of blood pressure to convulsions. He says, "It is well known that the blood pressure is a function of the intracranial pressure, rising directly with it. Normally the blood pressure is higher than the

<sup>1</sup>Ervin: Jour. Amer. Med. Assn., 1918, lxx, 1218.

intracranial pressure. The height of the blood pressure over that of the intracranial, or the difference between the two, is a margin of safety. As these two approximate each other, as the margin diminishes toward zero, or even tends to become a negative quantity (that is, when the blood pressure is less than the intracranial pressure) less blood is sent to the brain."

When this happens the brain must undergo some changes, one of which shows itself in fag of the vasomotor center. When this occurs the blood pressure which has been maintained as high as possible to counteract the high intracranial pressure, drops. The intracranial pressure now becomes greater than that of the blood, the pupils dilate, and the convulsion comes on. The muscular contractions of the muscles play a vicarious part in improving the condition by forcing blood from the periphery and increasing the amount sent to the brain. With this increased blood supply the centers take up their work again for the time being, and the convulsion passes.

Ervin shows by his observations upon his patients that a drug like nitroglycerine which will reduce the blood pressure will also tend to produce a convulsion if it is given at a time when the factor of safety is small, whereas a drug like adrenaline which will increase the blood pressure will tend to prevent or to shorten a convulsion if it is given at the appropriate time.

—P. G. W.

# *The Journal of Laboratory and Clinical Medicine*

VOL. III.

ST. LOUIS, SEPTEMBER, 1918

No. 12

## ORIGINAL ARTICLES

### RECENT ASPECTS OF STREPTOCOCCUS INFECTION

BY FREDERICK P. GAY, MAJOR, M.R.C., U. S. ARMY.\*

NO group of bacteria can claim more varied and more numerous types of proved and possible pathogenic activity than the streptococci. Although these microorganisms are readily recognized and usually easily isolated their mode of infection is often so unsuspected and their manifestations so protean that only in the recent years have their possibilities of harmful action been realized. An attempt is made in this review to collect and correlate the extensive literature, the bulk of it American in origin, on this subject that has appeared in recent years. The incentive to this collection has been the occurrence of the severe epidemic form of streptococcus bronchopneumonia and empyema which has occurred in army cantonments during the past few months.

Streptococci occur in water, dust, and milk, but their presence there is perhaps more accidental than has usually been assumed (Broadhurst). At all events our interest is greater in the fact that these organisms are present regularly in the alimentary canal of man and the domestic animals and that they are associated directly or indirectly in numerous human and animal diseases.

Any consideration of the streptococci, whether from the point of view of their habitat, or of the epidemiology, pathogenesis and specific therapy of disease at once involves discussion of the unity or multiplicity of the existent species. The successive discoveries of streptococci in apparently causative relation to such diverse disease processes as erysipelas, puerperal fever, endocarditis, rheumatic fever, general septicemia and septic sore throat would suggest at least

\*From the John Herr Musser Department of Research Medicine, University of Pennsylvania. Under the direction of Division of Medicine and Related Science, National Research Council. Published by permission of the Surgeon-General.

that there are many allied though similar specific strains. Recent work on experimental streptococcus infection would indicate a delicately adjusted selective affinity on the part of individual strains of the organism, in consonance with what is observed under natural disease conditions. It seems at least logically possible that two strains of a given microorganism which are identical by all morphological and cultural criteria currently employed may differ sharply in respect to the diseases they produce. We know certainly that two such strains do differ quantitatively in virulence.

It is, however, of first importance in attempting to separate streptococci into groups to detect constant and reliable morphologic or physiologic differences between them and it may at once be admitted that earlier attempts of this sort were unsatisfactory. The size or appearance of the individual cocci, their arrangement in pairs rather than chains, the length of the chains themselves, and the growth in ordinary media, have all been realized to represent variations which may lie within the life history of any pure strain that is naturally or artificially submitted to differences in environment. We shall direct our attention solely to the more recent methods of classification which have been shown to be of more constant and significant value.

#### CLASSIFICATION OF THE STREPTOCOCCI ON THE BASIS OF PRODUCTION OF A HEMOTOXIN (HEMOLYSIN)

The most significant property possessed by certain of the streptococci is that of dissolving red blood cells. The fact that this property is confined to certain types of streptococci and not possessed by others serves not only as a method of classification among streptococci but also, at least to some extent, bears a definite relation to the pathogenic properties of the hemolytic strains. Marmorek<sup>1,2</sup> (1895), who was the first to work systematically with the problem of streptococcus immunization, believed firmly in the unity of the streptococcus, basing his opinion largely on the uniform properties which his cultures showed. These properties were first of all the ability of all his human pathogenic strains to produce lysis of red blood cells as evidenced by the hemorrhagic exudate in the bodies of infected rabbits. Secondly, he utilized as a criterion of unity the inability of any strain of streptococcus to grow in the filtered culture fluid in which any other streptococcus had grown and lastly he found that an antiserum against a given strain of streptococcus neutralized the infectivity not only of that particular strain but of all the other strains of streptococcus with which he was working. This property of producing lysis of red blood cells in the animal body possessed by human pathogenic strains of streptococcus was further noted by Bordet in 1897 and in 1901 Besredka found that the filtrates of streptococcus cultures likewise had the property of dissolving red blood cells in the test tube. It was not, however, until 1903 that Schottmüller suggested that this hemolytic property be used as a means of classifying the streptococci.

By smearing human pathogenic strains of streptococcus on 40 per cent blood agar plates, Schottmüller found that these organisms readily fall into three definite groups in accordance with their effect on the blood medium, the characteristics of each of which are briefly as follows:



1. Number of human strains, particularly those derived from acute and severe generalized infections such as erysipelas, puerperal infection, scarlet fever and empyema are differentiated in their growth by producing a clear zone, 2 to 3mm. in diameter, surrounding each individual colony from which the red blood cells disappear. This hemolytic zone varies slightly in degree with individual strains of the organism but unmistakably and clearly separates them from streptococci belonging to the other two groups. This first type of hemolytic streptococcus Schottmüller designated as *Streptococcus longus* or *erysipelatis*. It is since currently referred to as *Streptococcus hemolyticus*.

2. The second type of streptococcus is characterized by the reduction of hemoglobin in the red blood cells surrounding the colony, with the formation of a greenish coloration from which the name of *Streptococcus viridans* or *mitior* was chosen for this group of organisms. The *Streptococcus viridans* occurs characteristically in subacute human infections as in abscesses, endocarditis and meningitis. The designation of "mitis" or "mitior" indicates the relatively slight virulence of this organism both for man and experimental animals as compared with *S. hemolyticus*.

3. The third class of streptococcus of human pathogenic significance is indifferent in its action on red blood cells; is characterized by a slimy mucoid growth, is capsulated and was designated *Streptococcus mucosus*. This organism occurs particularly in certain severe cases of lobar pneumonia and generalized infections. Although this organism was originally described as a streptococcus by Howard and Perkins, more recent studies (Longcope and Hanes) have shown that it should with few exceptions (Dochez and Gillespie) undoubtedly be classified among the pneumococci on the basis of bile solubility, inulin fermenting properties and immunity reactions. It forms the Type III pneumococcus of present classification.

Rosenow in 1904 independently drew attention to the blood plate method as a means of separating the pneumococcus from the streptococcus since the former produces an effect identical with the *Streptococcus viridans* in blood and the particular strains of the streptococcus with which Rosenow worked were all sharply hemolytic. In 1906 Ruediger found the strains of streptococcus which he isolated from scarlet fever cases all produced definite hemolysis and noted moreover that such strains were more virulent for rabbits than the *viridans* cultures. Since these preliminary observations increasing significance has been given to the absence or presence of hemolysis in blood cultures of streptococcus as a means of classification. In conjunction with the sugar fermentation tests to be later considered it is found that hemolysin production forms the first step in a satisfactory basis of classification of the known types of streptococcus which have been encountered. It seems fairly certain that the property of hemolysin production is constant in any given strain as shown by the work of Anthony and more particularly by that of McLeod. The ultimate classification of streptococci, particularly of human pathogenic strains, is immediately simplified and rendered intelligible by a primary classification on the basis of hemolysis as has been shown by the work of Thro, and more recently by Holman and by Blake. Certain modifications in the original blood plate method used by Schottmüller have

been suggested. Lyall,<sup>1,2</sup> for example, tests hemolysin production by adding a suspension of defibrinated sheep corpuscles to broth cultures. Smith and Brown and Smillie prefer to use poured blood plates using deep colonies for the detection of the true hemolytic strains (their Beta type of streptococcus). The majority of observers, however, prefer the superficial streak method proposed by Schottmüller as giving a more uniform, rapid and characteristic result. Practically the only recent workers with streptococcus who have failed to obtain fully successful results with the blood plate method are Hopkins and Lang. These authors found that all stages of variation exist between true hemolytic strains and the viridans strains but as Blake has pointed out it is probable that their results are to be explained by the fact that their observations extended over several days' growth of the cultures instead of being made at the end of 24 hours as is the usual method, and that their medium was not uniform in composition.

Various bloods have been used in detecting hemolytic streptococci. Those most frequently employed are human, horse and rabbit. Becker who has made comparative studies with blood from several species gives reasons for regarding human blood as superior to others. In this connection it should be noted that Breton has found that the order of increasing susceptibility of different bloods to destruction runs as follows, rabbit, man, horse, guinea pig, goat and ass. Considerably smaller percentages of blood than that proposed by Schottmüller ranging from 5 to 20 per cent have been found most satisfactory. It should be noted at this point that the presence of a fermentable sugar (e. g. dextrose) in blood agar prevents hemolysis (Ruediger,<sup>2</sup> Lyall<sup>2</sup> and Davis). Hemolytic strains grown under these conditions resemble methemoglobin or viridans strains, and the green pigment in the latter is increased in the presence of sugar (Aschner). Ruediger<sup>3</sup> was apparently incorrect, however, in assuming that methemoglobin production by the viridans strains is due to acid production for Blake<sup>2</sup> has shown that it takes place in presence of a buffer mixture of primary and secondary potassium phosphates, where neutrality is maintained. The latter writer has also demonstrated that the action of viridans takes place only in presence of oxygen and consists in the reduction of oxyhemoglobin to methemoglobin.

Some work has been done with the nature of the hemolytic substance produced by streptococci by Ruediger,<sup>2,4</sup> by Besredka, by Breton and by Lyall. The hemolysin is best formed in cultures when serum is added to the medium. The hemotoxin is destroyed by heating to 56° for 30 minutes (Lyall). According to Lyall the hemolysin seems definitely associated with the bacterial bodies rather than in solution. Opinion would differ as to the antigenic properties of this toxic substance. Breton and Tchitchkine claim to have produced an antistreptolysin in rabbits whereas Ruediger and Besredka both failed. It would appear from McLeod's work that hemolysin production in the body of susceptible animals is distinctly greater than is produced in the test tube.

No little interest and importance attaches to the relation of hemolysin production to the pathogenicity of streptococcus strains. Whereas it is certain that the majority of the most characteristic pathogenic human strains are hemolytic it would seem the opinion of many observers from Schottmüller on that there is no direct quantitative relation between the grade of hemolysin production and

the pathogenicity of any given organism, e.g. Floyd and Wolbach, Lyall,<sup>2</sup> Marmorek<sup>1,2</sup> who was first to observe hemolysin production regarded it as very definitely associated with virulence not only qualitatively but quantitatively. Linsgelsheim holds the same opinion. The entire question has recently been reviewed by McLeod whose consideration of the problem would seem at once more direct and convincing than that of previous observers. McLeod in reviewing the literature on the subject finds that 16 authors have expressed disbelief in any direct relation between hemolysin production and virulence; 6 authors have expressed a neutral attitude whereas 9 believe there is a definite relation between the two properties in streptococcus and in 7 additional publications there is indirect evidence which would seem to confirm the latter point of view. McLeod brings out the important experimental point which would seem to have been overlooked by most observers which is that any observation which proves or disproves the relation of hemolysis to pathogenicity must be based on hemolysis demonstrated primarily *in vivo* in the particular animal concerned in the virulence test. The author concludes from his own observations that the degree of hemolysin production in the animal body is closely related to virulence. As a basis for estimating virulence for man of human strains of streptococcus he proposed determination of the grade of hemolysis produced on human red blood cells in suspension.

The majority of authors who have used the blood plate method as a means of primary differentiation between strains of streptococci have proceeded further to classify these strains both on the hemolytic and nonhemolytic side by the employment of carbohydrate tests originally introduced by Gordon.<sup>1,2,3</sup> Schottmüller regarded all hemolytic strains as belonging to a single group and such is the opinion of Blake who has offered the most recent classification. Holman, however, whose classification is certainly more comprehensive, divides the hemolytic strains of streptococci on the basis of fermentation tests into eight groups. There can be no question that such groups exist and may be regarded as fairly constant. It is true, however, as Blake has pointed out that at least four of these hemolytic groups of Holman are qualitatively of minor importance, containing as they do a very small number of representative strains and showing no particular relation in any instance to a specific disease process. As further evidence of the unity of various hemolytic strains in spite of certain differences in their fermentation of carbohydrates may be mentioned the fact that they would seem to agree in such immunity tests as alexin fixation (Howell, Kinsella) in agglutination tests and also in cross anaphylactic tests in the experiments of Davis.<sup>2</sup>

The effect of streptococci on blood may serve as a further means of classification apart from the simple separation of hemolytic and nonhemolytic strains. Under the nonhemolytic strains it is found that certain organisms reduce oxyhemoglobin to methemoglobin producing green coloration of the viridans cultures to which reference has been made. These green pigmented strains of streptococci on blood may be separated from those that produce no effect on the medium. Holman classifies both the methemoglobin and the indifferent strains together, although he divides the hemolytic strains into eight varieties. On the other hand Lyall would make two separate groups in accordance with these peculiarities.



## CLASSIFICATION OF STREPTOCOCCI ON THE BASIS OF FERMENTATION OF THE SUGARS

Differential sugar media were first used in classifying streptococci by Gordon<sup>1,2,3</sup> (1903-1905) who used seven different substances for this purpose; lactose, saccharose, raffinose, inulin, salicin, coniferin and mannite. He tested 300 strains of streptococci and obtained many types. This work was extended by Andrews and Horder (1906) who correlated the studies of Gordon and of Houston<sup>1,2</sup> and on the basis of the study of 1200 strains classified streptococci into 6 more or less definite groups all of which are represented in the more modern classification, for example, in that of Holman. Since the pioneer studies of Gordon and of Andrews and Horder numerous attempts have been made to classify streptococci on the basis of carbohydrate fermentation alone. These attempts have included varying numbers of strains from different sources and the results so far as simplification or correct understanding of the actual varieties of streptococci are concerned, are confusing. This confusion is due first of all to the failure of the majority of workers to introduce a primary separation by the blood plate method and secondly to individual variations in technic. The original types identified by Andrews and Horder have for the most part been recognized but their significance and relative importance in the hands of each observer have varied markedly. The importance of classification from the standpoint of human pathogenesis should rest primarily on the relation of particular strains to some human disease process and it must be admitted that such identification is not at the present time possible. We recognize, however, that the hemolytic types are particularly concerned with the more severe and acute disease processes. There may also be some relation between fermentation reactions alone and such a process but they are certainly not so definite. Among those investigators who have relied entirely on fermentation tests in classifying streptococci may be mentioned Buerger, Winslow and Palmer, Bergey, Fuller and Armstrong, Stowell, Hilliard and Schleisinger, Hopkins and Lang and Broadhurst.<sup>1,2</sup>

Before proceeding to the more simplified and satisfactory classification which makes use of the blood plate method followed by carbohydrate differentiation, note should be made as to the more important criteria by which carbohydrate fermentation has been studied with an effort to point out the most recent and satisfactory methods in vogue. In the first place it was evident from much of the early work that failure to demonstrate carbohydrate fermentation depended in no small part on the unsuitableness of the culture medium employed in demonstrating the results. Buerger attempted to improve this deficiency by adding ascitic fluid to the carbohydrate broth solution a method which was later employed by Thro<sup>1,2</sup> who noted, however, the possibility of introducing an easily fermentable sugar such as dextrose in this fluid. Broadhurst<sup>3</sup> obtained better results by using a meat infusion broth than when meat extract broth was employed. Holman<sup>2</sup> has suggested a very satisfactory medium containing dilute bovine serum added to the carbohydrate dissolved in meat extract broth. It is obvious that no authentic results can be obtained unless the organism is given favorable conditions for growth.

The method employed in determining acid production again has varied in the hands of different observers. Litmus has been employed by several, notably



by Bergey and Hopkins and Lang. An attempt at greater accuracy in the demonstration of finer differences in the amount of acid produced by titration of the culture fluid with phenolphthalein as an indicator has been employed by Winslow and Palmer, Fuller and Armstrong, Broadhurst, Smith and Brown and Blake. It is doubtful, however, if the variations noted give information of importance. Some of the more recent observers, notably Holman<sup>3</sup> and Henrici have employed Anrade's indicator instead of the original litmus to indicate acid production.

Carbohydrate fermentation in the majority of instances is demonstrated in a 1 per cent solution of the sugar in question added to a sugar free broth but almost always in fluid media although some observers have used agar slants for the purpose.

In our opinion the most satisfactory classification of streptococci is that of Holman which consists in a primary differentiation of hemolytic and nonhemolytic strains by the superficial streak method on blood agar followed by classification on three sugars; lactose, mannite and salicin. All sugar tests are made in a meat extract sugar broth to which has been added dilute sterile bovine serum. Incubation from 3 to 5 days is made and the acid production is indicated by Anrade's indicator, included in appropriate amount, in the original extract broth. Holman has studied a large number of strains isolated by himself and others and has correlated his findings in the general scheme of classification which he offers, so far as possible with those of previous investigators. As a result he finds that 16 types of streptococci may be recognized, 8 hemolytic and 8 nonhemolytic. Under the nonhemolytic are included true viridans cultures as well as those which have no effect upon the blood medium. Lyall, as already mentioned, would prefer in his classification to further subdivide the nonhemolytic strains into methemoglobin and indifferent strains and such a classification may be utilized if desirable. A careful inspection of Holman's groups of streptococci shows as Blake has pointed out that many of them may be judged to be of minor importance from the numbers of actual strains that have been found. Nor is the relation of any one of these minor groups to any particular disease process striking. If we omit the least important of these groups we find ourselves confronted with six major strains of streptococci, 2 hemolytic and 4 nonhemolytic, and it is interesting to note that these strains are precisely those originally described by Andrews and Horder. They are presented in the following table which includes hemolysin production, their principal fermentation reactions and their origin as derived from Holman's tables. (See Table 1.)

#### CLASSIFICATION OF THE STREPTOCOCCI ON THE BASIS OF IMMUNITY REACTIONS

The various immunity reactions so frequently employed for diagnostic purposes such as agglutination, anaphylaxis and alexin fixation give the most convincing evidence of biological grouping. Separate strains of bacteria, for example of pneumococci, although indistinguishable by cultural methods may be allocated in separate groups by appropriate reactions of this sort. These results are available, moreover, not only for the purposes of classification but form the necessary prelude to intelligent attempts at specific therapy. The bulk of the work on immunologic classification of streptococci has been done in connection with

TABLE I  
THE MORE IMPORTANT STREPTOCOCCI IN HOLMAN'S CLASSIFICATION

	HEMOLYSIS	LACTOSE	MANNITE	SALICIN	ORIGIN
<i>S. pyogenes</i>	+	+	—	+	Nose and throat, pus, blood.
<i>S. anginosus</i>	+	+	—	—	Nose, throat (scarlet fever) blood, endocarditis, septicemia, epidemic sore throat.
<i>S. fecalis</i>	0	+	+	+	Human and animal feces, milk, rheumatism, endocarditis.
<i>S. mitis</i> or <i>viridans</i>	0	+	—	+	Nose and throat, human and animal, tonsils, pyorrhea, endocarditis, abscess, rheumatism.
<i>S. salivarius</i>	0	+	—	—	Throat, pyorrhea, milk.
<i>S. equinus</i>	0	—	—	+	Feces (horse and man) throat (?) urine, pyorrhea.

the study of antistreptococcus serum. It has dealt largely with the question of unity or multiplicity of the streptococci in general and as associated with disease entities. For the most part these particular studies have not been preceded by any consideration of hemotoxin production or fermentative reactions. For this reason we shall consider at this point only immunological tests as related to the more purely biological grouping of streptococci and leave the more extensive relations of pathogenicity as related to antigenic properties to a later section.

Swift and Thro attempted to demonstrate the relations of several strains of streptococci to one another by carefully testing cultures of each strain with a corresponding series of antisera each prepared by immunization with a single one of these strains. They were dealing for the most part with viridans cultures and found that agglutination and conglutination reactions did not serve to separate their strains from one another although specific for streptococci. Fixation reactions on the contrary seemed to show that most strains produced a separate immune body, in other words that viridans strains were largely heterogeneous. Similar results with a more extensive series of cultures have since then been obtained by Kinsella and Swift and by Howell. Kinsella and Swift were able to make three general groups of the viridans strains which tend to overlap. Howell similarly finds also that there are less specific fixations among the viridans than among the hemolytic group but that strains from specific disease entities tend to fall together. She finds further no correlation between fixation reactions and fermentation. In unpublished experiments Kinsella has found that the hemolytic strains group together in fixation.

Several less definite conclusions are drawn by other earlier observers of the agglutination reaction. Floyd and Wolbach in a limited series found a correlation between fermentative reactions and agglutination and fixation reactions. Kliger concluded that the agglutination test is of no value in classifying streptococci. Krumwiede and Valentine regarded viridans strains as heterogeneous on the basis of agglutination tests. Smith and Brown and others have used agglutination

tests successfully to demonstrate relationship between organisms isolated from different cases in a given epidemic of streptococcus infection. Certain of these observations on the agglutination reaction in less experienced hands may be open to suspicion of technical inaccuracy, for the streptococcus is particularly difficult to work with satisfactorily.

Davis<sup>2</sup> has attempted to classify streptococci by the anaphylactic test by injecting a series of guinea pigs with a given strain and attempting intoxication of each one in the series with a separate strain 14 to 26 days later. His conclusions would seem to agree with those obtained from fixation reactions, namely, that there is no cross sensitization between hemolytic and viridans cultures; hemolytic strains, however, interact.

These immunity tests so far as carried out seem to agree with biochemical reactions and indicate that the hemolytic group of streptococci are closely allied and are separate from the viridans strains. Viridans strains are presumably more heterogeneous. The last and most conclusive type of experiment in connection with other purely biological characteristics remains to be tested. Will a given strain of a certain group of streptococcus when used for the production of an active immunity protect against itself only or against several or all strains of that group? Will the serum of such a protected animal transfer passive immunity against the homologous strain only or against a number of strains? These questions again are ones which will concern us primarily in discussing immunization against the streptococcus.

#### MUTATIONS IN STREPTOCOCCI

Much confusion in the question of ultimate classification of the streptococci, as indeed in the classification of other bacteria, arises from the debated question of mutation. All progress in bacteriology so far as relating a given type of bacterium to a certain disease has depended on what would seem a justifiable confidence in the constancy of bacterial species. Each successive refinement in technic enables us still further to subdivide organisms which are practically a single species, as in the case of the pneumococcus and the differentiation between the paratyphoid bacilli and the typhoid bacillus, but all evidence is that each one of these types or varieties is relatively fixed not only in its cultural characteristics but also in its pathogenic properties. On the other hand it is evident to all experienced bacteriologists that minor variations in the physiological properties of bacteria occur with natural or artificial changes in their environment. Among such changes may be mentioned the increase or decrease in the property of fermenting sugars and a rise and fall in pathogenicity. In the case of streptococci the growth in short or long chains with a corresponding production of turbidity or sediment in broth are familiar instances. We have already referred to the production of hemolysin by certain streptococci and the reduction of oxyhemoglobin to methemoglobin by the viridans strains as being relatively fixed characteristics which may be utilized as a basis of classification. It is true that these properties may be lost under certain unfavorable conditions as for example in the case reported by Ruediger who by growing hemolytic streptococci in glucose broth for two years found that they lost the property of clearing blood agar. Anthony reports that only 95 per cent of the hemolytic streptococci re-



main true to type. Smith and Brown mention a slight reduction in hemolytic property in some of their strains.

More fundamental changes in streptococci are claimed by other observers. For example, Buerger and Rytenberg claim to have isolated encapsulated hemolytic streptococci which by passage through white mice became transformed into a pneumococcus. Rosenow<sup>2</sup> reported in 1912 that he was able to transform the streptococcus epidemicus, a capsulated organism, by growth on agar into streptococcus pyogenes and by regrowth on milk to restore its original condition. In a later publication<sup>3</sup> he has reported many more fundamental changes; by a series of passages through experimental animals he claims to have transferred 17 strains isolated as viridans into pneumococci and 21 strains of hemolytic streptococci into viridans and some of them further into pneumococci. Eleven strains of pneumococcus were likewise made to correspond to hemolytic streptococci. Davis<sup>2</sup> has also reported a few instances in which he was able to transform a typical hemolytic streptococcus into a streptococcus mucosus and also the reverse. Aschner has more recently described a transfer of pneumococcus into viridans strains and a viridans strain into the hemolytic variety. Although no such fundamental mutations are claimed, Broadhurst in her environmental studies on streptococci, found that certain rather striking differences in fermentation reaction could be produced in microorganisms by subjecting them to "living" environmental factors as for example to fresh milk, saliva and intestinal extract. She records these changes as more varied and constant than the simple physiological changes produced by unfavorable surroundings and the presence or absence of suitable food stuffs.

The more profound type of mutation such as those suggested by Rosenow may well shake our confidence in current bacteriological methods if they prove to be usual. Experience, however, indicates that such changes if they do occur under natural conditions must be most exceptional and as Smith and Brown expressed it they do not under natural conditions proceed rapidly enough to interfere with current bacteriological methods. Certain fundamental objections have been raised to the experimental production of these mutations which are briefly as follows: In the first place any mutation based on the injection of a given streptococcus into a normal animal and subsequent isolation of a different type of streptococcus may be due to the isolation of a streptococcus normally present in that animal's body rather than the one injected. It has been shown by Holman<sup>4,5</sup> that streptococci and pneumococci occur frequently in the tissues and even the blood of guinea pigs and their isolation from animals which have been inoculated with another type of organism may readily lead to overgrowth of the normal inhabitant and the supposition that it represents a mutation of the injected organism.

In the second place we must consider whether or not we are dealing in the first instance with pure-line cultures as has been outlined by Cole and Wright. In other words we must consider whether in dealing with an apparently pure culture we are dealing with the multiplication of a single organism or with a mixture of several biotypes some of which, endowed with specific properties, increase relatively rapidly under one set of environmental conditions at the expense of the others. The only method of avoiding what is really an impure culture



would be isolation of a single cell by the Barber method or might be effected by several successive isolations from a single colony.

#### HEMOLYTIC STREPTOCOCCI IN DISEASES INVOLVING THE THROAT AND RESPIRATORY TRACT

The hemolytic streptococcus of Schottmüller corresponds to the organism with the pathogenic properties originally assigned to *S. erysipelatis* (Fehleisen), *S. pyogenes*, or *S. longus* (v. Lingelsheim). It is the microorganism usually found in the severe and often rapidly fatal septic processes, in general septicemia, in puerperal fever, in erysipelas and in many forms of angina or sore throat. It is, usually pathogenic for laboratory animals, particularly for rabbits and mice, although this property will be found to fluctuate within considerable limits. It is my purpose in this review to deal rather with the more recently recognized or emphasized hemolytic streptococcus infections than with the older and more usual forms.

Hemolytic streptococci are intimately associated either directly as causative agents or indirectly as secondary invaders with a number of diseases which involve the throat and respiratory tract. As we shall see in further detail streptococci are frequently the apparent cause of sporadic and epidemic sore throat, of bronchopneumonia and empyema and are associated usually as secondary invaders in such diseases as scarlet fever and measles which involve the upper respiratory tract. The relation of streptococci to these infections is complicated by the fact that the streptococcus is a normal inhabitant of the nose, throat and tonsils. The exact percentage of normal or average individuals who show streptococci is somewhat doubtful and estimates have varied all the way from 1 to 100 per cent. Earlier observations in this regard are of little value because no classification of streptococci on the basis of hemolysis was given and even later estimates are marred by failure to specify what type of streptococcus was found. Ruediger was perhaps the first to make a careful estimate of hemolytic streptococci and of pneumococci in the normal throat as compared with a series of cases in scarlet fever. He found that 59 per cent of normal throats showed hemolytic organisms in small numbers whereas a considerably larger percentage showed the pneumococcus. It is probable that with the criteria then available the pneumococcus was confused with the streptococcus viridans. A recent estimate by Smillie would put the occurrence of streptococci in average throats, which is meant to include a number of individuals suffering from minor disturbances such as chronic tonsillitis, at 50 per cent. It seems probable through internal evidence, since Smillie used the Smith-Brown criteria of hemolysis, that at least a large percentage of these colonies were streptococcus viridans (his Alpha type) as seen in deep colonies. He found the true hemolytic streptococcus (his Beta type) present in only 1 per cent of average throats. At all events we conclude that streptococcus viridans is more frequently present than the hemolytic streptococcus in normal throats and when the hemolytic form is present it occurs in relatively small numbers as contrasted with large numbers in certain of the diseases which we are about to discuss. A further possibility arises, namely, that the type of hemolytic streptococcus present in pathological conditions differs from

that present in normal strains; a possibility by no means as yet sufficiently verified to be worthy of much consideration.

*Streptococci in Epidemic Sore Throat.*—Numerous epidemics of septic sore throat have been noted in England since 1875, and have been admirably summarized by Winslow and by Savage. During the years 1911 and 1912 three different foci of this disease appeared in the United States; followed in the years 1913 to 1915 by several other smaller epidemics. In May, 1911, 1,034 cases with 38 deaths occurred in and about Boston which have been described by Winslow, and later by Smith and Brown. In December of the same year a large number of cases, possibly 10,000, occurred in Chicago which have been reported by Capps and Miller and Davis and Rosenow in particular. In February and March of 1912 between 1,000 and 3,000 cases with about 30 deaths occurred in Baltimore. These have been reported by Hamburger, by Leutscher and later by Frost, Stokes and Hatchel. In 1913 several epidemics occurred in New York State as reported by North, White and Avery and by Krumwiede and Valentine,<sup>2</sup> and later in 1915 at Mount Vernon reported by Winslow and Hubbard. Similar smaller epidemics also appeared in the surrounding areas of the original epidemics about Chicago (Capps and Davis; Rosenow and Moon) and Boston, (Smillie). These later epidemics were apparently of considerably less severity than the original ones.

This particular disease which has been designated as septic sore throat differs at least in intensity very markedly from the ordinary follicular tonsillitis. It is accompanied by extreme prostration and in the more severe epidemics by a group of serious and often fatal complications. Among these may be mentioned peritonsillar abscess, otitis media, empyema, arthritis, nephritis, peritonitis, erysipelas, endocarditis and meningitis. The death rate is by no means slight as indicated by the figures already given.

From the very first and even in the earlier English epidemics a distinct relation was noted between these cases and the milk supply. As early as 1903 Pierce found moreover that there seemed to be a relation between the septic sore throat and the occurrence of mastitis or garget in the cows that furnished the milk. In the American epidemics the milk supply came at once under suspicion for it was found that the cases were ordinarily confined to the route supplied by a single dairy. It was also noted that at the farms from which this milk appeared there might be cases of garget among the cows and in some instances a number of cases of sore throat were found among those who handled the milk.

Hemolytic streptococci have, in all the recent epidemics, been immediately found both in the throats of those ill of the disease, in the lesions of the fatal cases and also in the milk supplied to them. Descriptions of this particular hemolytic streptococcus have in general varied little in the hands of different observers. The organism has been uniformly hemolytic although the degree of hemolysis as described varied somewhat probably depending on the type of blood agar plates used for differentiation. The Chicago investigators (Davis and Rosenow, Rosenow,<sup>2</sup> Davis<sup>3</sup>) at first described the regular occurrence of a capsule surrounding the organism which they isolated and were inclined to regard it as intermediate between streptococcus mucosus and streptococcus pyo-

genes. It is evident, however, that this "*Streptococcus epidemicus*" as they at first called it can not be regarded as a distinct variety, for Rosenow found that the original strains when grown on agar lost their capsule and subsequently regained them readily when grown for a single generation in milk. Leutscher who also found a capsule did not regard this as sufficient criterion on the basis of which to separate this organism from *S. pyogenes*. Davis,<sup>4</sup> in his most recent publication, regards the organism as identical with streptococcus pyogenes. At all events we know that capsules frequently appear in the animal body and may be associated with the degree of virulence possessed by the organism in question (Bordet; Bail and Kleinhans). The capsule originally described may perhaps better be referred to as capsular substance (Smillie).

The origin of this particular hemolytic streptococcus, that is so readily distributed by means of milk and with such serious effect has given rise to considerable discussion and can not yet be regarded as having been definitely settled. Streptococci of several varieties occur normally in perfectly clean and certified milk. The streptococcus lacticus for example, a nonhemolytic variety has been regularly found by Ruediger<sup>5</sup> and Heinemann.<sup>1,2</sup> Hemolytic streptococci also occur in milk that is apparently healthful but its presence at once suggests or would apparently prove that there is some disease of the udder in the cow that furnished such milk. (Ruediger,<sup>5</sup> Mathers, and Davis<sup>5</sup>). It has further been recognized for many years (Nocard and Mollereau) that mastitis or garget in cattle is due to a streptococcus. The occurrence, moreover, of cases of mastitis in cows furnishing milk that has given rise to these epidemics is frequent though by no means invariable and suggests a bovine origin of the human cases. Two opinions have arisen as to the origin of the streptococcus actually concerned in the human disease. The first and older opinion is that it is caused by a streptococcus of bovine origin which has first caused mastitis in the affected cows; the second and more recently maintained opinion is that it is due to a human strain of hemolytic streptococcus derived from the throats of milkers and accidentally propagated in the cow's udder or inoculated in the milk.

It has been proved by Savage, by Davis and Capps and by Mathers that cases of true mastitis or at all events closely resembling mastitis may be produced in goats and in cows by injecting human strains of hemolytic streptococci into the milk ducts or even by a slight abrasion of the teats. Savage on the other hand failed to produce human sore throat by means of bovine streptococcus strains. There is apparently some difference of opinion as to whether the disease produced by Davis and Capps is really true bovine mastitis or not. It was so regarded by the authors in their original communication but Smith and Brown referred to it as a mild inflammatory reaction and Davis and Capps in their more recent communication would apparently accept this latter definition. At all events they have proved beyond doubt that human hemolytic strains may live in the udder of cows for a considerable period of time. It is further pointed out by Smith and Brown that the ordinary garget of cattle which occurs throughout the year could not explain the epidemic form of human tonsillitis which occurs only at certain seasons.

The crucial proof, however, rests in the identity or nonidentity of human bovine hemolytic strains as derived from mammitis in conjunction with an epi-



demio of sore throat and on the other hand from the patients suffering from sore throat. Certain differential points have apparently been demonstrated. Smith and Brown and Davis<sup>9</sup> both agree that the human strains are virulent for rabbits whereas the bovine strains are not. Davis has shown that the human pathogenic strains are less resistant to heat than the bovine strains. Smith and Brown have pointed out certain differences in the sugar tests although Broadhurst<sup>2</sup> found no such criteria on which to base a separation between her two groups of cultures.

Smillie in the most recent article on this subject has found that sporadic cases of sore throat also show the same type of hemolytic streptococcus as the epidemic cases and probably differ from these latter cases only in mode of distribution. In this connection he further points out that carriers of the hemolytic streptococci may retain organisms for several months after recovery. Efficient pasteurization (Rosenow and Moon) is apparently sufficient to destroy the streptococcus in milk. It is also destroyed by the souring of milk (Davis)<sup>6</sup> and the organisms moreover do not increase notably at the ordinary temperature at which milk is handled.

*Streptococci in Scarlet Fever.*—Streptococci were observed in smears from the angina of scarlet fever as early as 1884 by Loeffler, and have since then been isolated and studied with more and more exact methods by several observers. Ruediger for instance found hemolytic streptococci in the tonsils constantly while the inflammation of the throat was active and noted moreover that they decreased rapidly in numbers with the subsidence of the throat symptoms. Some of these organisms fermented mannit and others did not and they showed interagglutinations with a specific serum from goats immunized with one of the strains. Anthony found hemolytic organisms almost constantly in throats of scarlet fever cases and in the heart's blood of 10 of 18 autopsies in fatal cases. It was also present in the complications of scarlet fever. Smillie found that the presence of the true hemolytic streptococcus (his Beta type) was almost constant in the throats of the severer cases of scarlet fever but less likely to occur in the milder cases. It was also persistent in many instances for several months in recovered scarlet fever cases and is thought by him to be a possible means of transmitting, not scarlet fever, but septic sore throat, believing as he does in the identity of the strains isolated from the two conditions.

Streptococci are by no means so frequently found in the blood of scarlet fever cases although often present in the fatal cases. Moser found them in 63 of 93 cases at death but rarely during life; Hektoen found them in only 12 per cent of those examined and then only in the more severe cases. That it is probably the usual cause of death in scarlet fever would seem certain from these findings which are corroborated by other observers. The general opinion is, however, that the streptococcus is not the cause of scarlet fever and it has been specifically shown by Landsteiner that the injection of scarlet fever strains of streptococcus in apes did not produce scarlet fever although the blood of scarlet fever cases transmits the disease to these animals.

Moser and von Pirquet found that scarlet fever streptococci were agglutinated by the serum of animals immunized against these organisms and of scarlet fever patients but that normal throat streptococci were not so affected. Weaver



found that streptococci derived from scarlet fever cases were agglutinated by the serum in such cases but also by the serum of cases from other types of streptococcus infection. Weaver mentions normal serum controls but does not include them in his protocols. The whole question is open in view of the statement of Zelenski that most streptococci *are agglutinated by normal human sera*. Tunnicliff found a rise in opsonic index to the streptococcus during the course of scarlet fever. Besredka and Dopter were unable to obtain a fixation reaction with the streptococcus and the serum of scarlet fever patients. Moser and others have utilized an antistreptococcus serum obtained by immunizing horses with scarlet fever strains and by its use reduced the mortality in scarlet fever in a series of cases as compared with untreated controls. Although of no particular bearing as regards the relation of streptococci to scarlet fever it may be noted that Weaver<sup>2</sup> and others have obtained beneficial results in scarlet fever by injecting the serum of recovered cases.

*Streptococci in Smallpox.*—Several observers have found streptococci as a general blood infection in cases of smallpox, whereas others have failed to demonstrate the organism in this connection. Perkins and Pay found the organism regularly in all fatal cases in the blood stream and in many cases that were not fatal. They do not specifically state whether their organisms were of the hemolytic variety or not. They point out that it is not to be assumed that the streptococcus has any etiological relation to the disease. It is interesting to note that their observations lead them to assume that the portal of entry is through the bronchial mucous membrane which agrees with the approved or supposed mode of entrance in this group of the acute exanthemata which we are considering.

*Streptococci in Measles.*—The hemolytic streptococcus has been found quite frequently and often regularly in the throat of measles cases. Anthony for example found the hemolytic variety of the organism in the throat of every one of 24 cases, and the viridans was also noted to be present. Levy and Alexander found hemolytic streptococci in 77.1 per cent of 388 cases of measles. In connection with the etiological study of bronchopneumonia which we shall consider presently Lorey noted that the hemolytic streptococcus was the invariable cause of all complications in measles and was fatal when found in the blood stream. Among the most frequent of these complications are otitis media and bronchopneumonia, the latter of which will concern us more specifically in a moment.

Quite apart from the finding of the hemolytic streptococcus in the respiratory tract in measles as an incidental infection is the recent description by Tunnicliff<sup>2,3</sup> of the regular occurrence of a viridans variety of streptococcus in the blood stream of early cases both of measles and of rubella. In 42 out of 50 cases Tunnicliff has found streptococci more particularly during the preeruptive stage of the disease, and the organism on first isolation and not infrequently in subsequent cultures is anaerobic. She further noted that the serum of the patients concerned had opsonins with specific effect upon the organisms isolated from the particular form of disease in question, that is to say measles serum reacted with the true measles strains and not toward the rubella strains or the reverse. It is obvious that this organism may be suspected of having some direct etiological relation to

measles and is quite distinct from the hemolytic streptococcus considered above.

*Streptococci in Bronchopneumonia and Empyema.*—It was noted by Eyre in 1910 that the streptococcus is the most frequent organism found in cases of bronchopneumonia following measles. It is practically the only organism present in those cases of bronchopneumonia which follow diphtheria. In 1913 Thursfield described the streptococcus as regularly present in measles—bronchopneumonia. In this connection may be noted with interest experiments on various types of pneumonic lesions in dogs by Wollstein and Meltzer.<sup>1-2</sup> Lamar and Meltzer had produced a typical lobar pneumonia in dogs by means of intrabronchial insufflation of cultures of pneumococcus. Winternitz and Hirschfelder later by the same method produced entirely analogous results in rabbits. Following these pneumococcus experiments Wollstein and Meltzer employed cultures of the streptococcus with equally striking but somewhat different results. The mortality was distinctly less in animals that had received the streptococcus cultures and the lesions were distinctive and resembled human bronchopneumonia. Particular interest attaches to the description that is given of the interstitial infiltration of cells in this experimental bronchopneumonia in view of the later description in connection with camp epidemics described by MacCallum. It was found that various strains of streptococci varied only in the degree and extent of lesions produced and not in the type of lesions.

During the past few months attention has been drawn to a serious and epidemic form of bronchopneumonia which has occurred in the army cantonments throughout the United States. This pneumonia at first confused with lobar pneumonia due to the pneumococcus has chronologically succeeded it and in the earlier cases was often combined with it. It has been unquestionably proved that this type of pneumonia is due to the streptococcus. In a large number of cases, although by no means invariably, this bronchopneumonia has been a direct sequel of measles and in many of them perhaps one-third of the total has been followed by empyema with a high mortality rate. It appears that this form of fatal empyema has been well recognized by pediatricians who have met with it in connection with the bronchopneumonia following measles in children. Koplik found that in a series of cases of empyema in children from one to five years of age, that 15 per cent were due to the streptococcus and 69 per cent were due to the pneumococcus with or without streptococcus. It may be assumed that with present methods of differentiation between these two organisms distinctly more than 15 per cent of his cases might have been shown to be of pure streptococcus origin. These cases of empyema followed measles, scarlet fever and whooping cough. Zybelle states that the streptococcus is the most frequent cause of empyema in young children although after the age of five other organisms appear with greater frequency.

As already mentioned the earlier cases of bronchopneumonia and empyema in army camps due to the streptococcus followed chronologically on the usual cases of lobar pneumonia which had occurred from the month of October, 1917, onward, and which were gradually succeeded in numbers and finally replaced by the streptococcus group. In the several reports available of this severe bronchopneumonia in camps in Illinois, Virginia, Texas and Michigan hemolytic streptococci have been found in all or nearly all instances of the disease. Now that it has been

fully studied post mortem it may be clearly differentiated from lobar pneumonia although it was by no means readily separable from it by clinical methods. The particular microorganism concerned both in the empyema fluid and in the tonsils, sputum and lungs of these cases have been studied by Dick, Irons and Marine, Alexander, Levy and Alexander, Cole and MacCallum, Cumming, Spruit and Lynch, and others. Alexander found in his early observations that apparently two types of hemolytic streptococcus were present differing in their fermentative properties on mannit and corresponding thereby to the types described by Holman as streptococcus infrequens and streptococcus pyogenes respectively. Kendall who has made a special study of 58 strains of hemolytic streptococcus derived from cases in a number of army camps in Illinois, Virginia, Texas and Michigan under the auspices of the National Research Council found that about 80 per cent would correspond to the pyogenes strain and 20 per cent to the streptococcus infrequens. Ultimate decision, however, as to whether one or several strains of hemolytic streptococcus are concerned in these cases must be left to further investigation. Immunological studies should in particular be undertaken on this problem.

The epidemiology of these camp epidemics of bronchopneumonia and empyema are very instructive even in the present incomplete state of our knowledge concerning them. In the first place it may be noted although it is perhaps a matter of no great importance in the transmission of the disease, that Alexander found the same microorganism in nasal swabs from four sick horses in Camp Zachary Taylor at the time when bronchopneumonia was at its height. In this connection the description by Mathers<sup>2</sup> of the occurrence of equine influenza or shipping fever at various stock yards in the United States, a disease well recognized to be due to the streptococcus since the time of Schutz, is of interest. In 117 cases Mathers<sup>2</sup> isolated hemolytic streptococci from the nasal discharges and various tissues of the body in fatal cases. The disease is manifested by a high fever, nasal discharge and frequently such complications as pneumonia, pleurisy, empyema and arthritis. The lungs are always affected in fatal cases. Of far greater importance are the epidemiological studies in the camps themselves of positive factors such as dust and the actual presence of streptococci in the throats of normal and diseased individuals. Several studies have been made as to the presence of the hemolytic streptococcus in the general camp population. It has been assumed from the work of Smillie that hemolytic streptococci are present in only a small percentage of normal individuals. Cumming, Spruit and Lynch found about 6 per cent of carriers of *S. hemolyticus* (their Type III) in Fort Sam Houston. Fox and Hamburger and Levy and Alexander both reporting from Camp Zachary Taylor, found about 15 per cent normal carriers of the hemolytic streptococcus as an average. Irons and Marine in Camp Custer report as high as 70 per cent of the individuals as harboring these microorganisms. In regimental units that have been found particularly susceptible to the bronchopneumonia as high as 89 per cent (Levy and Alexander) were found to harbor hemolytic streptococci. In the average of measles cases taken at various periods during the course of the disease from one-third to two-thirds show streptococci in their throats (Cumming et al, 35 per cent; Cole, 36.5 per cent; Levy and Alexander, 77.1 per cent).



The observations by Cole and his colleagues as regards ward contamination in measles cases have been most enlightening and of great practical importance. In a relatively small series of cases Cole found that whereas measles cases on their entrance to the hospital showed the presence of hemolytic streptococci in their throats in only 11.4 per cent that this average rose rapidly in mixed wards so that in the interval from the 8th to the 16th day after admission the percentage of positive carriers had risen to 56.8 per cent. This observation and the following study reported by Levy and Alexander have led to a most important measure of preventing the spread of this serious infection. Careful measures of segregation were undertaken at Camp Zachary Taylor and positive streptococcus carriers among the measles cases were promptly separated from those who did not show the organism in their throats on admission. After these measures were carefully carried out it was found that 36.8 per cent of the positive cases showed complications, following measles attack whereas among those in whom no streptococci had been found complications occurred in 6.4 per cent only. The complications following measles in the Camp Taylor cases occurred in 30.6 per cent of the cases. In the Fort Sam Houston cases Cumming, Spruit and Lynch report that 49 per cent of those who were known to harbor the true hemolytic streptococcus showed complications, whereas a control series that contain other streptococci (their Types I and II hemolysis) showed complications in only 13 per cent of the cases. The obvious relation between the presence of a hemolytic streptococcus in the throat and subsequent complications proved to be due to this organism is therefore well demonstrated.

Numerous complications have occurred in these camp measles cases among which may be noted in approximate order of frequency, bronchopneumonia, acute tonsillitis, acute bronchitis, acute suppurative otitis media, empyema and pericarditis, and then a series of local manifestations at places more remote in the body such as erysipelas, adenitis, peritonitis and meningitis. We are particularly concerned at this point with the cases of bronchopneumonia and still more interested in those cases followed by empyema. Bronchopneumonia has occurred in about one-third of all the cases of measles that have been carefully described and perhaps a third of these cases have been followed by empyema (Levy and Alexander, 34 per cent). It should be noted in this connection that although the diagnosis of empyema may be more readily assured, the diagnosis of bronchopneumonia, particularly as differentiated from lobar pneumonia, has been by no means so simple or certain. It is further to be noted that many cases and indeed groups of cases, as for example at Camp Lee, Virginia, have borne no relation to preceding measles and again that empyema has occurred not infrequently without antecedent bronchopneumonia or at least without bronchopneumonia which was detected. Owing to the incompleteness of the reports and necessary inaccuracies in diagnosis, no reliable figures could be given at the present time as to the proportion of each of these types of disease.

The course of the bronchopneumonia following adult measles has been carefully studied and reported on by Cole, Fox and Hamburger, Irons and Marine and others. The disease usually begins with rapid symptoms of prostration followed by respiratory disturbances, and frequently is accompanied by angina. The physical signs of involvement of the lung tissue are often indefinite and a diagnosis is



difficult even with the aid of fluoroscopic examination. The bronchopneumonia when followed by empyema may be transitory or may persist for a considerable period of time. If the involvement of the lung subsides the patient soon shows less evidence of prostration although the involvement of the pleura may rapidly increase.

On postmortem examination the lesions have been found in various localities to differ somewhat in extent and distribution, in accordance with the mode of distribution of the streptococci in the body. The lung lesions due to the streptococcus alone have been in most cases described as similar or identical to lobular pneumonia as found in children. In many instances lobar pneumonia has occurred simultaneously with the lobular pneumonia as for example in the Sam Houston cases described by MacCallum and in those cases described by Dick and in many such instances it has been possible to demonstrate the presence both of the pneumococcus and the streptococcus. Apparently two methods of distribution of the streptococci have been observed, the less frequent method in the form of a septicemia in which case the microorganism is found more regularly in the circulating blood and various lesions in the peritoneum, meninges and joints as well as in those structures directly adjacent to the lungs. In a recent small group reported by Lucke multiple subserous hemorrhages and jaundice likewise occurred. A large number of cases is apparently due to a spread of streptococcus infection by extension down the respiratory passages as in those cases described by MacCallum and also mentioned by Irons and Marine and Hamburger and Mayer. In these cases the blood cultures during the course of the disease are rarely positive; in the Fort Sam Houston cases for example only 13 per cent according to Cole were positive and 17 per cent according to Cumming, before death.

The lung lesions have been described in detail by MacCallum who has found that the form of bronchopneumonia differs somewhat from that usually described in children which consists of an exudate into the alveoli. The exudate in these post measles cases was largely interstitial in character and would apparently correspond to that described in the experimental streptococcus pneumonia in dogs by Wollstein and Meltzer. Small grayish nodules extending above the cut surface of the lung were found by MacCallum in many cases resembling tubercles. In his group of cases the spread of the disease seemed definitely by extension down the respiratory passages through the lungs and into the pleura and this corresponds to the cases we have observed at Camp Lee. Lucke has differentiated on gross appearance four types of bronchopneumonia in his series from Camp Zachary Taylor the first two of which, characterized by the peribronchial distribution and general distribution in all lobes, he regards as characteristic of measles bronchopneumonia. The adjacent tissues particularly the pericardium are not infrequently involved and in the less usual and metastatic form of the infection the joints, peritoneal cavity and the meninges may present secondary foci of infection.

Pleuritis and empyema which as noted occurred with varying degrees of lung involvement and with varying duration of symptoms of bronchopneumonia is the serious and often fatal complication in this syndrome. The mortality of the cases of empyema has ranged from 23 per cent in those cases described by Hamburger

and Mayer in which the ultimate results were by no means available, to 46 per cent in the cases described by Irons and Marine at Camp Custer and 47 per cent in the cases described by Cole and MacCallum. In the fatal cases the duration of the pleurisy and subsequent empyema has varied from a few days to several weeks and recovery has been in most cases extremely prolonged and indefinite. The empyema fluid as first observed is serous, often bloody or brownish in character, and only gradually becomes purulent. The definite increase of pleural exudate is accompanied by compression and atelectasis of the lung and the extent of the fibrin and the thickness of the pleura varies with the duration of the process. It is not within our province to discuss the methods of treatment which have been suggested and are being tried in dealing with this severe and often fatal complication. It would appear to be the growing consensus of opinion that surgical interference is best postponed until the subsidence of the acute symptoms of bronchopneumonia with the relative improvement of the patient which follows and also until the pleural exudate has become distinctly purulent in character.

The modes of prevention of this bronchopneumonia empyema complex whether following measles or not are again matters which are still in the process of being evolved. Most significant are the observations of Cole and of Levy and Alexander as to the spread of infection in the presence of positive carriers whether in normal individuals or in those attacked by measles. The system of the separation of the clean from the dirty measles cases as described by Levy and Alexander has proved most satisfactory in diminishing the incidence of complications. Levy and Alexander find that even with careful observation of the cubicle system, and care against contamination by obvious means, that when dirty and clean cases are mixed in the wards the streptococcus infection will spread from one individual to his neighbor readily. In one ward in which 12 clean and 12 dirty patients were placed at the end of a week only 3 noncarriers remained. Efforts to disinfect the mouth in the positive carriers have not been successful. Levy and Alexander found that in spite of gargles and sprays and with various solutions 71.7 per cent of their measles cases discharged from the hospital still retained the streptococcus hemolyticus in their throats. The few reports that have been made on the possibilities of specific therapy by means of vaccines and serums in these epidemics have been largely in the line of suggestion. It is obvious that any progress in this direction must be preceded by more fundamental studies on the nature of streptococcus immunity than have as yet been undertaken.

#### STREPTOCOCCUS VIRIDANS IN CONNECTION WITH THE RHEUMATIC FEVER GROUP OF INFECTIONS AND IN OTHER DISEASES

In his earlier differentiation between the hemolytic and viridans forms of streptococci Schottmüller pointed out the pathogenic significance of these two types of organisms. Whereas the hemolytic streptococci are concerned more particularly with acute, severe, and generalized infections the streptococcus mitior or viridans was found particularly in association with the milder, more chronic group of infections with certain definite localizations. It has been noted from the time of Schottmüller and Ruediger that viridans strains are less pathogenic for experimental animals than hemolytic strains.

There is a clinical group of diseases associated with certain forms of tonsillitis which have long been recognized and which comprise acute articular rheumatism or rheumatic fever often with coincident infection of the joints which may become chronic; this rheumatism is frequently complicated by endocarditis and associated not infrequently with chorea. It would appear that not only tonsillitis but also pyorrhea may serve as the local starting point of this generalized infection (Cecil, Moray). Westphal, Wassermann and Malkoff isolated a streptococcus from a case of rheumatic fever, chorea and hyperpyrexia and produced with this organism inflammation of the joints in rabbits. This observation has been followed by many others including much experimental consideration of the apparently similar diseases which may be produced in animals. Different conclusions have been drawn particularly as to the specificity of the lesions produced by the streptococcus strains from rheumatic fever. Menzer, Cole and Rothschild and Thalheimer have shown that streptococci from various other sources would produce similar lesions. Poynton and Paine isolated a form of streptococcus viridans to which they gave the name of "Diplococcus rheumaticus" because they regarded it as being specific and different from other forms of streptococci, a viewpoint also held by Beattie. We shall discuss in a moment the experimental production of arthritis in animals in a more general connection and for a moment confine ourselves to the occurrence of the streptococcus in human disease.

Although the streptococcus viridans may be found not infrequently in rheumatic fever with polyarthritis it is apparently not of regular occurrence to judge from the recent work of Swift and Kinsella who obtained it from the blood stream in only 8.3 per cent of fifty-eight cases. They failed, moreover, to isolate it from the joints in any case. In subacute endocarditis on the other hand, so frequently associated with polyarthritis, Kinsella<sup>2</sup> obtained either the viridans or an anhemolytic saprophytic streptococcus with regularity and proved the existence of specific antibodies for the strain concerned in the serum of the patient. The viridans has been found by Cecil in 16 out of 23 cases of mild tonsillitis of the recurrent type so frequently associated with rheumatic fever. Quigley found viridans in 10 of 21 cases of chorea in the circulating blood.

*Elective Affinity of Streptococci.*—The diversity of opinion as to the specificity of experimental arthritis produced by streptococci from rheumatic fever and by strains from other diseases has been again brought into great prominence through Rosenow's work on tropism or the elective affinity of bacteria. There is proof from the immunological work of Kinsella, Krumwiede and Valentine and others that the viridans organisms are a heterogeneous group and their association with varied and strictly localized chronic lesions in the body gives color to this opinion. The specific reasons why localization of apparently similar organisms should take place now in one part of the body and now in another may be explained in two general ways. First, by a lowering of resistance of the affected part in the individual (*locus minoris resistentiae*) and secondly by a specific affinity of the particular culture in question for a given tissue in the body. It is, of course, logically possible that both these factors may be operative in producing localization of lesions. Rosenow has been led to emphasize the organotropic effect of the bacteria rather than any particular diminished resistance in the part affected. In an elaborate series of experiments extending over several years, em-



ploying very large numbers of experimental animals, Rosenow has apparently been able to show that streptococci obtained from particular lesions and in all instances presumably the cause of those lesions have, when first isolated from the body, a particular affinity for the tissues whence they originated. Thus Rosenow and his coworkers have shown from the standpoint of etiology that streptococci are not only present in rheumatic fever, and in endocarditis, pericarditis and myocardial lesions as well as the joints affected in this disease complex, but also in such apparently diverse affections as appendicitis, (Rosenow and Dunlap, Rosenow);<sup>4</sup> ulcer of the stomach and duodenum, (Rosenow);<sup>5</sup> Cholecystitis, (Rosenow);<sup>6</sup> erythema nodosum, (Rosenow),<sup>7</sup> Herpes zoster, mumps, (Herb, Rosenow and Dunlap); myositis, iritis, (Rosenow);<sup>8</sup> iridocyclitis (Irons, Brown and Nadler). Each of the strains of streptococcus viridans isolated from one of these conditions will on injection intravenously in rabbits or dogs tend to localize in the same tissue and produce the same lesion in a relatively high percentage of animals as compared with the percentage of such lesions in animals inoculated with nonspecific strains of streptococcus. These specific relations are readily visualized in (Table II) which is taken from Rosenow's<sup>9</sup> more elaborate tabula-

TABLE II  
ELECTIVE LOCALIZATION OF STREPTOCOCCI (ROSENOW)

ORIGINAL STRAIN FROM	Animals — 833. Strains — 220.	PER CENT LESIONS WITH SPECIFIC STRAINS
	PER CENT LESIONS WITH NONSPECIFIC STRAINS	
Appendix	5	68
Ulcer stomach	20	60
Cholecystitis	11	80
Rheumatic fever	27 Joint	66
	14 Endocardium	46
	2 Pericardium	27
	10 Myocardium	44
Erythema nodosum	2 Skin	90
Herpes zoster	2 Skin	70
Mumps	0 Parotid	73
Myositis	10	35
Endocarditis	14	84

tion. It will be seen from this table that the results are frequently very striking as for example in the case of erythema nodosum; although nonspecific strains may produce the lesions in question the percentage is always higher with the specific strains. The burden of proof rests we believe with those who would disprove Rosenow's contention. In matters of this sort positive evidence, when obtained, is of more value than negative results, and failure to repeat does not disprove. Rosenow does not claim that his results are invariable or that nonspecific strains may not less frequently produce the same results. His argument is based on quantitative evidence and nothing but quantitative evidence of the same sort and in like volume would suffice to disprove his thesis.

Among the other recent observers who have produced and studied arthritis with viridans strains may be mentioned Cecil,<sup>2</sup> Jackson, and Faber. The latter author makes interesting observations which point to the factor of localized lowered resistance as operative in the lodgement of streptococci. He obtained arthritis regularly only when the successful inoculation had been preceded by sensitization



of the joint with a dead culture of the streptococcus and found that this sensitization is specific in that it is not produced by a staphylococcus for example. Chronic arthritis has been produced by Davis<sup>8</sup> in rabbits and by Schloss and Foster in monkeys. Among those who have recently emphasized the nonspecificity of viridans strains in producing arthritis may be mentioned Rothschild and Thalhheimer, Henrici, and Detweiler and Maitland. Moody in an interesting series of experiments in which he utilized strains obtained from cases of alveolar abscess with or without the complication of articular rheumatism found that the percentage of joints affected experimentally were the same in both series, thus indicating the absence of specific affinity in the pyorrhea-rheumatic strains. Rosenow<sup>10</sup> has emphasized the importance of using recently isolated strains in producing local lesions since they are found to lose their specific affinity on cultivation and even to some extent on animal passage. He has also emphasized from the beginning that variation in oxygen tension and other technical details which might well explain the failure of others to repeat his results. (Rosenow).<sup>10</sup>

Certain of the apparent etiological associations of streptococci with the diverse diseases we have categorically summarized in discussing Rosenow's theory, are worthy of much fuller consideration that we feel incumbent on us to give in this place. Rosenow and Dunlap, for instance, have pointed to epidemic conditions that may occur in the outbreak of appendicitis and of cases of parotitis which they felt could be traced to streptococci milk used in ice cream. The claimed etiologic relation of streptococci to cholecystitis, gastric ulcer, erythema nodosum, and herpes zoster were certainly unexpected and have naturally been received with some skepticism awaiting further confirmation. Rosenow<sup>4</sup> has further complicated his theory of elective affinity in the case of appendicitis by obtaining a summation of elective affinities on injecting mixtures of streptococci and colon bacilli, thereby apparently simulating natural conditions more fully.

*Streptococci in Anterior Poliomyelitis.*—In 1913 Flexner and Noguchi described a filter passing virus from tissues of cases of poliomyelitis and on cultivation apparently obtained a pure growth of globoid bodies in such filtrates which were regarded as the etiological agent in the disease. Until 1916 these results passed unquestioned. In this year a series of observers (Mathers,<sup>3</sup> Nuzum and Herzog, and Rosenow and Towne) found a somewhat peculiar form of streptococcus viridans regularly in the tissues of cases of anterior poliomyelitis. Since this time several investigators have concerned themselves with the etiologic significance of this microorganism. The coccus has been grown in pure culture from adenoids, tonsils, mesenteric lymph nodes, central nervous system and the spinal fluid in anterior poliomyelitis. There is apparently considerable variation in the cultures as regards size and shape of organism, pigment formation and also in its growth on various media (Rosenow and Wheeler, and Heist, Solis-Cohen and Kolmer).<sup>1-2</sup> An apparent correlation between these observations on cocci and the work of Flexner and Noguchi has been furnished by Heist, Solis-Cohen and Kolmer and also by Rosenow and Wheeler who by growing the coccus under anaerobic conditions have succeeded in obtaining a filterable form of the virus with bodies in the filtrate which resemble those described by Flexner and Noguchi. Of interesting confirmatory nature are the observations of Hektoen, Mathers and Jack-

son who have found cocci in stained sections of 50 cases of poliomyelitis obtained from different localities.

Considerable difficulty has arisen in connection with the experimental production of the disease with pure cultures of this coccus in animals. Whereas such experimental reproduction of the disease was described by Rosenow, it has been questioned by Bull who found no specificity of streptococci from cases of poliomyelitis as regards production of the lesions described by Rosenow in guinea pigs, dogs, cats and monkeys. Streptococci have been described in the normal tissues of the rabbit by Tsen in 7 out of 10 cases and of monkeys by Lamar who had found similar organisms in spontaneous septicemia. Bull found no lesions in his experimental animals resembling poliomyelitis and regards the streptococcus when present as a secondary invader.

Antibodies have been described in the serum of rabbits immunized against the streptococcus, particularly opsonins by Mathers and Howell who found further that these antibodies did not react with other strains of streptococci. Similar antibodies have been described in recovered monkeys by Rosenow and Gray. Patients suffering from poliomyelitis also contain opsonins for this particular form of coccus according to Heist, Solis-Cohen and Kolmer.<sup>2</sup> Most convincing of all in indicating the etiological relation of this microorganism to the disease are the results with an antiserum derived by immunizing horses with this form of coccus. Nuzum and Willy obtained such a serum which had curative effect on monkeys infected with poliomyelitis virus. Rosenow<sup>11</sup> has recently described curative results not only in monkeys but also in a series of human cases treated by an antiserum against the specific coccus. The injections were given intravenously and in 58 treated cases the mortality was 17 per cent. Of these seven out of the ten fatal cases were moribund at the time of first inoculation and if the mortality is corrected on the basis of 51 cases it is found to be only 6 per cent. A similar group of control cases, 23 in number, had a mortality of 35 per cent. The serum also left the early treated cases without any consequent paralysis, whereas this occurs regularly in about half the untreated cases.

#### IMMUNITY IN STREPTOCOCCUS INFECTIONS

Streptococci multiply in the human body in several different ways. They may rapidly proliferate invading the entire system and producing a form of septicemia against which form of activity the host offers apparently little resistance; they may localize at the point of entrance and be rapidly disposed of by means of leucocytes, or may persist, as for example in the pleural cavity and joints for a considerable time, later to assume more pathogenic properties and to invade the entire body in the form of septicemia; or again streptococci may be distributed as staphylococci are through the general circulation into metastatic foci as in the case of rheumatic fever, and with varying outcome. It is evident that in viewing the pathogenic activities of streptococcus we have to deal first with the proliferation of the microorganism itself and secondly with various toxins which that microorganism may form. In the case of the streptococcus we know that a substance which destroys red blood corpuscles is

formed by a majority of the highly pathogenic human strains. There is also some rather indefinite information as to formation of a leucocidin, a substance formed in greater amounts by certain of the staphylococci but also to some extent by the streptococci (Ruediger,<sup>6-7</sup> McLeod). There is no particular reason to believe that the hemotoxin *per se* has any predominant function in determining the result of the streptococcus infections, (Menzer<sup>2</sup>) although as we have already considered it apparently runs parallel with the degree of pathogenicity of any particular streptococcus. The work of Menzer further suggests that there may be some other type of toxin formed by the streptococcus which is responsible for the cachexia in animals who although they have succeeded in killing off the organisms themselves later succumb to a wasting process. We have, however, little precise information as to the nature of this toxin whether it be endo-or exo-cellular in origin (Weil).

Some interesting observations have recently been made by Hopkins and Parker concerning the disposition of streptococci when injected into the circulation of naturally resistant animals (cats) and nonresistant animals such as rabbits. They find as has previously been shown that the organisms disappear in both animals rapidly from the circulation. In rabbits they are destroyed in most organs but those which lodge in the muscles multiply there and afterwards reinvade the general circulation causing septicemia and death. It appears from their work that the fixed tissue cells are responsible for the destruction of the organisms in resistant animals rather than the fluids of the body. Leucocytes and bone marrow are apparently endowed particularly with the power of destroying streptococci according to Ruediger,<sup>6</sup> although the cooperation of opsonic substances in the serum is apparently necessary for complete destruction, at least in the less resistant animal. Apparently normal human and animal sera have little effect by themselves in destroying streptococci. Ruediger<sup>8</sup> also found that the serum of cold-blooded animals had no destructive action.

During the course of streptococcus infection in human beings and animals a certain definite reaction occurs in the body irrespective of the outcome of the infection. Whereas it may be true that ultimate destruction is due to the leucocytes or fixed tissue cells the cooperation of tropic substances is apparently always necessary. Whereas, the serum of human cases from streptococcus infection does not acquire the property of killing streptococci the whole blood does have this property according to Ruediger,<sup>7</sup> and the property is directly proportional to the number of leucocytes that are present. The opsonins or tropins may be shown to be actually increased in certain streptococcus infections as for example in subacute endocarditis as shown by Meakins. Fixation antibodies are apparently not usually formed in streptococcus infections. Besredka and Dopter, for example, found them absent in cases of scarlet fever although the streptococcus is frequently associated with the disease, and Hastings found them absent in cases of viridans infection. They were found not infrequently present in certain viridans infections, however, by Aschner provided the homologous organism was used as an antigen.

The occurrence of agglutinins in the serum of streptococcus infections has given rise to a great deal of discussion and has a bearing on the specificity of



certain strains of the streptococcus notably those from scarlet fever. Moser and von Pirquet found that the serum of cases of scarlet fever gave an agglutination reaction in low dilutions with streptococci derived from these cases. This reaction did not occur with other strains of streptococcus and apparently indicated the specificity of the strains derived from this disease. These results were apparently repeated by Hasenknops and Salge but Weaver at about the same time found that the reaction also occurred with the serum from other types of streptococcus infection. Kinsella obtained positive agglutinations and positive thread reactions with the sera of cases of subacute endocarditis and their respective streptococci. Wlassjewski agglutinated streptococci from cases of puerperal fever by means of the patient's serum. Zelenski, however, claimed that nearly all normal human sera could with proper technic be shown to agglutinate most strains of streptococci. None of these reactions can in the present state of our knowledge be regarded as of diagnostic value or as throwing any light on the classification of streptococci or their relation to any particular disease.

*Use of Vaccines in Preventing and Curing Streptococcus Infections.*—Von Behring reported in 1892, in brief, results obtained by him in collaboration with Knorr concerning the possibility of active and passive immunization against the streptococcus. These authors found that rabbits immunized against a given strain of streptococcus were protected against that particular strain and also against apparently different strains as determined by the inadequate criteria then available. Streptococcus vaccines have not, however, been used to any considerable extent as a means of protection against streptococcus infection. As a preliminary to the possibility of passive immunization against streptococci active immunization has been carried out repeatedly and with success, although the degree of resistance in the immunized animal is often slight irrespective of the properties which the serum possesses. We shall refer later to the difficulty of immunizing small animals against the streptococcus for the production of an antistreptococcus serum and may here simply note the observation of Moore that arthritis in rabbits which can so readily be produced with various strains of the streptococcus may be prevented in about 80 per cent of cases by the previous inoculation of killed cultures of the microorganism. Coincident with the interest in the therapeutic use of bacterial vaccines inaugurated by Wright many attempts have been made to affect human streptococcus infections advantageously by this means. They have been for the most part of little demonstrated value. It may be shown indeed that in human streptococcus infections as for example scarlet fever (Weaver and Tunnicliff<sup>1,2</sup>) and in infected rabbits (Weaver and Tunnicliff; and Simonds) that the tropic substances for the streptococcus rise after vaccine injections. The results in the treatment of human disease, however, by the means of vaccines are for the most part discouraging. They were found of no value by Boughton<sup>1,2</sup> in recurrent erysipelas even when galactose-killed streptococci, which were supposedly more advantageous were employed, and although the effect produced in erysipelas by these vaccines was at first regarded as favorable by Weaver and Tunnicliff they were later admitted to be of doubtful value by Weaver<sup>3</sup> himself. Weaver and Tun-



nicliff moreover obtained no significant results in treating complications of scarlet fever such as otitis media with streptococcus vaccines. Gabritschewsky suggested using streptococcus vaccine in scarlet fever and although he himself reports no definite results based on its use Langowoy who followed his suggestion apparently obtained some favorable effect in 154 cases. Davis<sup>9</sup> has reported a few cases of arthritis in which the vaccine apparently aided in a favorable outcome. Wright himself the arch supporter of vaccine therapy has quoted only a few cases of streptococcus septicemia treated by vaccines and these with very indifferent result. On the whole we must conclude that there is at present no conclusive evidence of the usefulness of vaccine therapy in streptococcus infections.

*Passive Immunization by Antisera in Streptococcus Infections.*—There is very little evidence in human or animal pathology which would lead us to suppose that recovery from streptococcus infection leads to any considerable or durable degree of acquired immunity. The well known susceptibility of certain individuals to repeated postmortem streptococcus infections, the recurrence of erysipelas and the like, all tend to evidence this fact. Von Lingelsheim has mentioned certain observations which tend to show the actual existence of acquired immunity in man. Neufeld failed to demonstrate protective substances for mice in the serum of recovered human streptococcus cases. We have already stated, however, that von Behring showed that rabbits could be actively immunized against the streptococcus and that this protection was transferable by means of the blood serum to other animals. It has also been suggested that Richet and Hericourt (1888) in demonstrating the first instance of passive transfer of immunity in dogs were dealing not with the staphylococcus as they imagined but with a streptococcus. Although these experiments of active immunization have been frequently repeated and very extensive studies have been made on the question of passive immunity by the serum of immunized animals much remains in doubt as to the best method of producing such a serum and its actual usefulness in combating streptococcus infection. Although large animals such as the horse and mule may be repeatedly injected with streptococci without great difficulty, much trouble has been experienced in immunizing small animals for the experimental study of this form of specific therapy. Among the most recent authors on this subject Hopkins and Parker have found that it is almost impossible to immunize rabbits by employing killed cultures of the streptococcus. Apparently the most successful method of immunizing consists in the use of living cultures rather than killed cultures as emphasized first by Neufeld and later by Weil and Schenk. Neufeld by means of one injection of a relatively large amount of washed killed cultures of streptococci followed by a second injection of a living, virulent and also washed culture produced an immune serum that was more potent in protecting mice than the serum of horses that had been immunized over a long period of time. Weil found that often a single dose of living culture produced a protective antiserum in rabbits whereas several doses of killed culture failed to do so. Similar results have been reported by Schenk who found that with doses of the streptococcus that eventually resulted fatally in rabbits, the serum of these animals

was strongly protective by passive transfer. Indeed the strength of the serum seemed to vary directly with the degree of infection.

The greater part of the extensive studies on the curative value of anti-streptococcus sera have naturally been carried out with the serum obtained from large animals particularly from horses and a number of different methods particularly in respect to the strains of streptococcus used for immunization have been utilized with very different results and it must be confessed with considerable resulting confusion as to what may ultimately be the method of greatest value. The variation in methods has depended first of all on the continued discussion as to the unity or multiplicity of streptococci, a matter obviously of prime importance in deciding on the type of antigen to be employed if successful therapeutic results are to be expected. In general there have been four different types of antistreptococcus serum produced varying in respect to the antigen employed: (1) a serum produced by means of a single strain of streptococcus or a group of strains derived from a given disease. This constitutes a so-called monovalent serum.

(2) A serum obtained by immunizing animals with varying numbers of different strains usually strains from different diseases. This constitutes a so-called polyvalent serum.

(3) A serum produced by immunization with original strains recently derived from human pathogenic processes.

(4) A serum obtained by immunizing animals with strains the virulence of which has been enhanced for animals (usually mice or rabbits) by means of repeated passage.

Various combinations of these methods of immunization have been employed by different authors. Results with each type of serum have varied enormously dependent on the observer and the type of cases treated. In few instances has it been possible to find consistent and convincing results even in a definite group of streptococcus infections in the hands of several observers with any given serum. The results, however, both in animal experiments and in treatment of human disease have been sufficiently encouraging to justify every effort not only along the lines already laid down but in further perfecting the methods of immunization now in vogue. We may now discuss the experimental results on which these various methods of immunizing procedure have been founded.

The earlier observers of transferred passive streptococcus immunity, von Behring and Marmorek, accepted and maintained the essential unity of streptococci or at least of the streptococci concerned in human disease processes and on this basis felt justified in using a monovalent serum prepared by means of a single strain. Marmorek to whom we owe the first extensive consideration of passive immunity by means of antistreptococcus serum proved to his satisfaction the unity of the streptococcus with which he was dealing by the existence of certain given characteristics: First the inability of any one of the streptococci to grow in the filtered culture fluid in which another of his strains had been propagated; secondly, a constant hemolytic property in all his strains of streptococci; it is evident that he was dealing with the strains of

the hemolytic streptococcus only. Third, and most important in this connection, the fact that the antiserum to a single strain of streptococcus was able to protect animals experimentally against infection with any of the other strains of streptococcus. A monovalent streptococcus serum essentially similar in type to that employed by Marmorek but with minor variations in each particular case has also been described by Aronson, by Zelenski, Menzer, Marxer and others. The belief of these authors in the essential unity of the streptococcus is based both on cross protection tests similar to those of Marmorek and agglutination of various strains of streptococcus other than the one used for immunization by the monovalent serum.

Van de Velde who was first to utilize agglutination tests with streptococci came to the conclusion by this means that there were numerous different varieties of streptococcus and for that reason advocated the employment of numerous strains from various sources in producing an immune serum designed to affect various streptococcus diseases. Agglutination reactions, cross fixation tests and absorption tests (Simon) in the hands of another group of observers following Van de Velde have likewise led to the conclusion that there are numerous varieties of streptococci. Among these observers may be mentioned Tavel, Moser and von Pirquet, Meyer and Simon.<sup>2</sup> This conception of the multiplicity of varieties of streptococcus is further complicated by the fact that certain observers for example Moser and recently Rosenow<sup>11</sup> have apparently proved variations in this microorganism by demonstrating a unity in the microorganisms derived from a given disease process. Moser and von Pirquet for instance found that the strains of streptococcus from scarlet fever reacted only with an antiserum obtained by immunizing with these strains, which serum had little effect on streptococci of other origin. Rosenow<sup>11</sup> and Mathers and Howell claim specific immune reactions and protective value in the serum of animals immunized against strains of streptococcus from cases of anterior poliomyelitis which apparently has little or no effect on streptococci of other origin. We have then in these latter cases a number of strains used to produce a given immune serum but the immune serum itself regarded as monovalent in respect to the disease from which its strains were derived.

The apparently directly contradictory results obtained by these two sets of observers, leading on the one hand to a belief in the essential unity of the streptococcus and on the other hand to its essential variety depend on technical variations and inaccuracies in the experimental methods pursued, and also and predominately to undoubted misunderstandings of the general nature of antigenic function involved. The technical difficulties and variations in performing agglutination tests with streptococci have been admirably reviewed by Zelenski in his contribution to the subject. Von Lingelsheim also discusses the matter. We can not at this time undertake a discussion of these complicated and important details but by passing to the second group of variations in the method of producing immune serum we may perhaps clarify the situation to some extent.

Two distinct types of bacterial antigens apart from single or multiple strains have been employed in producing streptococcus immune serum. Mar-

morek, Aronson and others have utilized strains of streptococcus that have been raised to a point of maximum virulence by repeated passage through mice or rabbits for purposes of immunization. These are the so-called "passage" strains. Tavel, Moser, Besredka and another group have on the contrary utilized strains of streptococcus recently derived from human lesions for injection. The use of these two different types must be understood to be quite apart from the employment of single or multiple strains and the resulting serum has been conclusively shown to vary distinctly in accordance with the original species of antigen employed. The use of human original strains by Tavel (1900) and later by Moser was selected largely on empirical grounds for the purpose of retaining supposed human pathogenic properties, but it became subsequently apparent that the choice was justifiable insofar as the anticipation of treating human disease. It appears from the work of Meyer, Simon,<sup>2</sup> and Hazenknops and Salge that an antiserum to virulent passage strains is effective not only against the particular strains employed but against any other homologous animal passage strain, irrespective of what the human disease origin of it may have been. In other words these passage strains have become animal-virulent and their antigenic properties give rise to antiserum capable of neutralizing this virulence. They are, however, not effective, according to Simon in neutralizing the infection of the actual human strains from which they were derived. The reverse has been demonstrated by Hazenknops and Salge who showed that freshly isolated human strains are agglutinated by the antiserum of Moser obtained by immunizing with fresh human strains but that the same strains after becoming virulent through animal passage are not affected by this serum. The obvious conclusion then is that bacteria for immunizing against the streptococcus for human needs should be used which retain so far as possible the original human pathogenic qualities, and which will therefore give rise to antibodies capable of neutralizing these properties. The difficulty as Simon has pointed out in attaining this goal rests in the fact that the original human strains are not very virulent for animals and according to this conception may not give rise to a good antibody production. It becomes evident that careful analysis of the existent methods is indicated, and that further experimentation is necessary to unravel further the complex question of antigenic properties if the ultimate solution of passive immunity to streptococcus infections is to be solved.

The mode of action of antistreptococcus serum in the cases where it is successful seems very definitely understood and generally agreed to. The fundamental observations of Denys and LeClef and of Bordet showed that antistreptococcus serum protected animals from streptococcus infection by so affecting bacteria that they were readily taken up and destroyed by white blood corpuscles. The importance of this indirect method of phagocytosis was further brought into prominence by the work of Wright on opsonins and the work of Neufeld and Rimpau on tropins. That streptococcus protection either in the normal or immunized animal is due to a dual mechanism of this sort, tropin and leucocyte, is generally accepted by all workers on that subject as for example by von Lingelsheim, Weaver<sup>4</sup> and Ruediger.<sup>7</sup> Another method of producing an immune substance for passage transfer and utilizing the tropic powers



of antistreptococcus serum to better advantage has been suggested by Caulfield who would use the pleural exudate of streptococcus immune animals for treatment rather than their serum. There is no evidence that the ordinary streptococcus serum includes an antihemolysin although the observation of Breton would indicate that its production is possible.

The usual method of testing the potency of antistreptococcus serum has been to employ mice or rabbits which are given the test culture dose with the serum administered either previously or simultaneously. It has little effect if administered any considerable period of time after the infecting dose of streptococcus. It may be possible when the proper antigenic preparation has been devised that some other method may be necessary.

At all events the ultimate object of antistreptococcus serum is to cure human or animal diseases due to this microorganism. Concerning this point the results are even more confusing and doubtful than those outlined in explaining the mechanism of streptococcus protection. It seems fairly definite that no striking results have been obtained in the majority of human streptococcus infections as for example in erysipelas, puerperal fever, rheumatic fever, arthritis and the like, by the use of antistreptococcus serum. We need not at this time attempt to summarize the results in any detail but it may suffice to state that the majority of observers record the curative value of antistreptococcus serum as negative or extremely doubtful. (See for example Besredka, Schwoner, Weaver,<sup>4</sup> Meyer). There are, however, at least two instances in which results have been claimed that are either markedly specific or at all events extremely encouraging and suggestive. In both instances the results have been obtained by methods utilizing the more modern form of immunization. Moser's results in scarlet fever cases form the basis of his figures which were carefully controlled by an untreated series of cases and must be regarded as extremely good. The treatment of poliomyelitis by Rosenow<sup>11</sup> and by Willy and Nuzum obtained with a serum directed against poliomyelitis strains is also extremely encouraging. LeCount and Jackson have apparently seen good results in preventing acute streptococcus nephritis in rabbits by use of specific serum.

#### CHEMOTHERAPY IN STREPTOCOCCUS INFECTIONS

In conclusion we may mention the attempts that have been made to affect streptococcus infections by chemotherapeutic means. In this connection the work of Churchman on the bacteriostatic action of dyes of the pararosanalin series particularly of gentian violet, on nearly all Gram-positive bacteria is of interest. Localized joint infections particularly gonorrheal in origin have been treated by lavage with gentian violet with some success by Churchman.<sup>2</sup> In our opinion, based on some unpublished work with the typhoid carrier condition in rabbits little is to be hoped from dyes of this category in more systemic or remote infections in the animal body. Although the pararosanalin series of dyes are highly bactericidal *in vitro* not only for Gram-positive but also for Gram-negative organisms like *B. typhosus* in the test tube, they are apparently without appreciable action when injected directly into the circulation of experimental animals. In addition to being very toxic in fairly dilute solutions

they become rapidly converted to leucobases in which form they are apparently inactive for bacteria.

In this connection should be mentioned the work of Hoffman, McClure and Sauer who found that dahlia, a mixture of methyl-violet and fuchsin, will retard the growths of streptococci in a dilution of 1 to 10,000 *in vitro* but is without effect in checking streptococcus infections in the animal body. Its action indeed was positively harmful.

Allison has tried several arsenic compounds, salvarsan, arsenol, and arsenobenzol, on experimental streptococcus infections in rabbits. When these substances are injected before the microorganisms are localized in remote tissues they seem to have a beneficial effect. They are said by the author to have been tried with some success also in human beings.

#### BIBLIOGRAPHY

(The references are listed by authors alphabetically. When a single reference to an author is quoted it is followed by no number: the first reference to each author when there are several also is followed usually by no number. Subsequent references to an author are numbered as they occur in the text.)

- Alexander, H. L.: Hemolytic Streptococcus Causing Severe Infections at Camp Zachary Taylor, Ky., Jour. Am. Med. Assn., 1918, lxx, 775.
- Andrews, F. W., and Horder, T. J.: Classification of Streptococci Pathogenic for Man, Path. Soc. of London, Lancet, London, 1906, pp. 171, 1245.
- Anthony, Bertha Van H.: Some Characteristics of the Streptococci Found in Scarlet Fever, Jour. Infect. Dis., 1909, vi, 332.
- Allison, C. S.: The Bactericidal Action of Arsenical Compounds on Experimentally Produced Streptococcal Septicemias, Jour. Med. Research, 1918, xxxviii, 55.
- Aronson, H.: Weitere Untersuchungen über Streptococcen, Deutsch. med. Wchnschr., 1903, xxix, 439.
- Aschner, P. W.: Studies in Pneumococci and Streptococci, Jour. Infect. Dis., 1917, xxi, 409.
- Bail, O., and Kleinhans, F.: Versuche über die Infektiosität von Strept. an Meerchweinchen, Zeit. für Immunitätsforsch., 1911, 1912, p. 199.
- Beattie, J. M.: Experimental Work in Relation to Micrococcus Rheumaticus and Streptococcus Pyogenes, Jour. Med. Research, 1906, xiv, 399.
- Becker, W. C.: The Necessity of a Standard Blood Agar Plate for the Determination of Hemolysis by Streptococci, Jour. Infect. Dis., 1916, xix, 754.
- Behring, E. von: Untersuchungsergebnisse betreffend den S. longus, Centralbl. f. Bakteriologie, 1892, xii, 192.
- Bergey, D. H.: Differentiation of Cultures of Streptococcus, Jour. Med. Research, 1912-13, xxvii, 67.
- Besredka, A.: Medicament Microbiens, Bibliotheque de Therapeutique Gilbert and Carnot; Balliere and Fils, Paris, 1907.
- Besredka, A., and Dopfer: Contribution de l'étude du rôle des streptococques au cours de la scarlatine, Annales Pasteur, 1904, xviii, 373.
- Blake, F. G.: (1) The Formation of Methemoglobin by Streptococcus, Jour. Exper. Med., 1916, xxiv, 315.
- (2) The Classification of Streptococci, Jour. Med. Research, 1917, xxxvi, 99.
- Bordet, J.: Contribution à l'étude du Serum antistreptococcique, Annales Pasteur, 1897, xi, 177.
- Boughton, T. H.: Injections of Homologous Streptococci Killed by Galactose in the Treatment of Suppurative Complications of Contagious Diseases, Jour. Infect. Dis., 1910, vii, 99.
- (2) Interaction of Serum Leucocytes and Bacteria in Phagocytosis as Observed in a Case of Recurrent and Relapsing Erysipelas, Jour. Infect. Dis., 1910, vii, 111.
- Breton, M.: De l'hémolysine produite par le streptocoque dans l'organisme infecté, Comptes rend. Soc. de Biol., 1903, lv, 886.
- Broadhurst, J.: (1) Environmental Studies of Streptococci with Special References to the Fermentative Reaction, Jour. Infect. Dis., 1915, xvii, 277.
- (2) A Biometrical Study of Milk Streptococci, Jour. Infect. Dis., 1912, x, 272.
- (3) The Effect of Meat and of Meat Extract on the Fermentative Activity of Streptococci, Jour. Infect. Dis., 1913, xiii, 404.

- Buerger, L.: The Differentiation of Streptococci by Means of Fermentative Tests, *Jour. Exper. Med.*, 1907, ix, 428.
- Buerger, L., and Rytenburg, C.: Observations upon Certain Properties Acquired by the Pneumococcus in the Human Body, *Jour. Infect. Dis.*, 1907, iv, 609.
- Bull, C. G.: The Pathologic Effects of Streptococci from Cases of Poliomyelitis and Other Sources, *Jour. Exper. Med.*, 1917, xxv, 557.
- Capps, J. A., and Davis, D. J.: The Relationship of Septic Sore Throat to Infected Milk, *Jour. Infect. Dis.*, 1914, xv, 130.
- Capps, J. A., and Miller, J. L.: The Chicago Epidemic of Streptococcus Sore Throat and Its Relation to the Milk Supply, *Jour. Am. Med. Assn.*, 1912, lviii, 1848.
- Caulfield, A. H.: Preliminary Report Upon New Methods for the Production of Anti-streptococcal Sera, *Jour. Path. and Bacteriol.*, 1916, xxi, 28.
- Cecil, R. L.: (1) Streptococcus Viridans in its Relation to Infections of the Upper Respiratory Tract, *Arch. Int. Med.*, 1915, xv, 150.  
(2) A Study of Experimental Nonhemolytic Streptococcus Lesions in Vitrally Stained Rabbits, *Jour. Exper. Med.*, 1916, xxiv, 739.
- Churchman, J. W.: (1) The Selective Bactericidal Action of Gentian Violet, *Jour. Exper. Med.*, 1912, xvi, 221.  
(2) Treatment of Acute Infections of the Joint by Lavage and Direct Medication, *Jour. Am. Med. Assn.*, 1918, lxx, 1047.
- Cole, R. I.: Experimental Streptococcus Arthritis in Relation to the Etiology of Acute Articular Rheumatism, *Jour. Infect. Dis.*, 1904, i, 714.
- Cole, R., and MacCallum, W. G.: Pneumonia at a Base Hospital, *Jour. Am. Med. Assn.*, 1918, lxx, 1146.
- Cole, L. J., and Wright, W. H.: Application of the Pure-Line Concept to Bacteria, *Jour. Infect. Dis.*, 1916, xix, 209.
- Cumming, J. G., and Spruit, C. B., and Lynch, C.: The Pneumonias: Streptococcus and Pneumococcus Groups, *Jour. Am. Med. Assn.*, 1918, lxx, 1066.
- Davis, D. J.: (1) Observations on the Growth of Streptococci in Blood Carbohydrate Medium, *Jour. Infect. Dis.*, 1917, xxi, 308.  
(2) Interrelations in the Streptococcus Group with Special Reference to Anaphylactic Reactions, *Jour. Infect. Dis.*, 1913, xii, 386.  
(3) Bacteriologic Study of Streptococci in Milk in Relation to Epidemic Sore Throat, *Jour. Am. Med. Assn.*, 1912, lviii, 1852.  
(4) The Relation of Streptococci to Bovine Mastitis and Septic Sore Throat, *Am. Jour. of Public Health*, viii, 40.  
(5) and (6) Hemolytic Streptococci Found in Milk, Their Significance and Their Relation to Virulent Streptococci of Human Origin, *Jour. Infect. Dis.*, 1916, xix, 236.  
(7) The Growth and Viability of Streptococci of Bovine and Human Origin in Milk and Milk Products, *Jour. Infect. Dis.*, 1914, xv, 378.  
(8) Chronic Streptococcus Arthritis, *Jour. Am. Med. Assn.*, 1913, lxi, 724.  
(9) The Etiology and Pathogenesis of Rheumatoid Arthritis, *Illinois Med. Jour.*, 1914, (September).
- Davis, D. J., and Capps, J. A.: Experimental Bovine Mastitis Produced with Hemolytic Streptococci of Human Origin, *Jour. Infect. Dis.*, 1914, xv, 135.
- Davis, D. J., and Rosenow, E. C.: An Epidemic of Sore Throat Due to a Peculiar Streptococcus, *Jour. Am. Med. Assn.*, 1912, lviii, 773.
- Denys and LeClef: Sur l'Immunité de lapin vacciné contre le streptocoque, *La Cellule*, 1895.
- Detweiler, H. K., and Maitland, H. B.: The Localization of Streptococcus Viridans, *Jour. Exper. Med.*, 1918, xxvii, 37.
- Dick, G. F.: A Bacteriologic Study of the Pneumonia Occurring at Camp Pike, Arkansas, *Jour. Am. Med. Assn.*, 1918, lxx, 1529.
- Dochez, A. R., and Gillespie: A Biologic Classification of Pneumococci by Means of Immunity Reactions, *Jour. Am. Med. Assn.*, 1913, lxi, 727.
- Eyre, J.: The Bacteriology of Bronchopneumonia, *Jour. Bacteriol. and Path.*, 1910, xiv, 160.
- Fäber, H. K.: Experimental Arthritis in the Rabbit, A Contribution to the Pathogenicity of Arthritis in Rheumatic Fever, *Jour. Exper. Med.*, 1915, xxii, 615.
- Flexner, S., and Noguchi, H.: Experiments on the Cultivation of the Microorganism Causing Epidemic Poliomyelitis, *Jour. Exper. Med.*, 1913, xviii, 461.
- Floyd, C., and Wollach, S. B.: On the Differentiation of Streptococci; Preliminary Notes, *Jour. Med. Research*, 1913, xxix, 493.
- Fox, H., and Hamburger, W. W.: The Streptococcus Epidemic at Camp Zachary Taylor, Ky., A Survey, *Jour. Am. Med. Assn.*, 1918, lxx, 1758.
- Frost, W. H.: Septic Sore Throat; A Milk-borne Outbreak in Baltimore, Md., Reprint from Public Health Reports No. 103, Government Printing Office, 1912.
- Fuller, C. A., and Armstrong, V. A.: The Differentiation of Fecal Streptococci by Their Fermentative Reactions in Carbohydrate Media, *Jour. Infect. Dis.*, 1913, xiii, 442.
- Gabritschewsky, G.: Ueber Streptokokkenvaccine und deren Verwendung bei der Drüse der Pferde und dem Scharlach des Menschen, *Centralbl. f. Bakteriol.*, I Abt. Orig., 1906, xli, 844.

- Gordon, M. H.: (1) 33d Annual Report of the Local Government Board, Report Medical Officer, 1903-04, p. 388.
- (2) Report Medical Officer, Local Government Board, Great Britain, 1910-11, xl, 302.
- (3) The Differentiation of Streptococci, Jour. Path. and Bacteriol., 1911, xv, 323.
- Hamburger, L. P.: An Epidemic of Septic Sore Throat in Baltimore and its Relation to a Milk Supply, Jour. Am. Med. Assn., 1912, lviii, 1109.
- Hamburger, W. W., and Mayers, L. H.: Pneumonia and Empyema at Camp Zachary Taylor, Ky., Jour. Am. Med. Assn., 1918, lxx, 915.
- Hanes, F. M.: An Immunological Study of Streptococcus Mucosus, Jour. Exper. Med., 1914, xix, 38.
- Hasenkops and Salge: Ueber Agglutination bei Scharlachs, Jahr. der Kinderheilk., 1903, lviii.
- Hastings, T. W.: Polyvalent Antigen for Streptococcus Viridans, Jour. Exper. Med., 1914, xx, 72.
- Heinemann, P. G.: (1) The Pathogenicity of Streptococcus Lacticus, Jour. Infect. Dis., 1907, iv, 87.
- (2) Significance of Streptococci in Milk, Jour. Infect. Dis., 1906, iii, 173.
- Heist, G. D., Solis-Cohen, M., and Kolmer, J. A.: (1) Studies in Epidemic Poliomyelitis. I. The Isolation and Cultivation of the Globoid Bodies, Jour. Infect. Dis., 1918, xxii, 169.
- (2) Studies in Epidemic Poliomyelitis. III. Comparative Studies of Cocci Isolated from Poliomyelitis, Jour. Infect. Dis., 1918, xxii, 182.
- Hektoen, L.: Bacteriologic Examination of the Blood During Life in Scarlet Fever with Special Reference to Streptococci, Jour. Am. Med. Assn., 1903, xl, 685.
- Hektoen, L., Mathers, G., and Jackson, L.: Microscopic Demonstration of Cocci in the Central Nervous System in Epidemic Poliomyelitis, Jour. Infect. Dis., 1918, xxii, 89.
- Henrici, A. T.: The Specificity of Streptococci, Jour. Infect. Dis., 1916, xix, 572.
- Herb, J. C.: Experimental Parotitis, Arch. Int. Med., 1909, p. 201.
- Hoffman, W. H., McClure, W. B., and Saner, L. W.: Simultaneous Injections of Streptococci and Dalia in the Guinea Pig, Jour. Infect. Dis., 1916, xviii, 353.
- Holman, W. L.: (1) The Classification of Streptococci, Jour. Med. Research, 1916, xxxiv, 377.
- (2) A Method of Making Carbohydrate Serum Broth of Constant Composition for Use in the Study of Streptococci, Jour. Infect. Dis., 1914, xv, 209.
- (3) The Use of Decolorized Acid Fuchsin as an Acid Indicator in Carbohydrate Fermentation Tests with Some Remarks on Acid Production by Bacteria, Jour. Infect. Dis., 1914, xv, 227.
- (4) The Invasive Quality of the Streptococci in the Living Animal, Am. Jour. Med. Sc., 1917, cliii, 427.
- (5) Spontaneous Infection in the Guinea Pig, Jour. Med. Research, 1916, xxxv, 151.
- Hopkins, J. G., and Lang, A.: Classification of Pathogenic Streptococci by Fermentative Reactions, Jour. Infect. Dis., 1914, xv, 63.
- Hopkins, J. G., and Parker, J. T.: The Effect of Injections of Hemolytic Streptococci on Susceptible and Insusceptible Animals, Jour. Exper. Med., 1918, xxvii, 1.
- Houston: Report London C. Council July 11, 1905. (2) Research Metr. Water Bd., London, 1910.
- Howard, W. T., and Perkins, R. G.: Streptococcus Mucosus Pathogenic for Man and Animals, Jour. Med. Res., 1901, vi, 163.
- Howell, K.: Complement Fixation of Streptococci, Jour. Infect. Dis., 1918, xxii, 230.
- Irons, Brown and Nadler: Localization of Streptococci in the Eye, Jour. Infect. Dis., 1916, xviii, 315.
- Irons, E. E., and Marine, D.: Streptococcal Infections Following Measles and Other Diseases, Jour. Am. Med. Assn., 1918, lxx, 687.
- Jackson, L.: Experimental Streptococcal Arthritis in Rabbits, Jour. Infect. Dis., 1913, xii, 364.
- Kendall, Arthur: A Summary of a Study Undertaken for the National Research Council up to May 23, 1918. Letter to R. M. Pearce.
- Kinsella, R. A.: (1) Unpublished Communication.
- (2) Bacteriologic Studies in Subacute Streptococcus Endocarditis, Arch. Int. Med., 1917, xix, 367.
- Kinsella, R. A., and Swift: A Classification of Nonhemolytic Streptococci, Jour. Exper. Med., 1917, xxv, 877.
- Kliger, I. J.: A Study of the Correlation of the Agglutination and the Fermentation Reactions among the Streptococci, Jour. Infect. Dis., 1915, xvi, 327.
- Kotlik, H.: Empyema in Infants and Children; its Frequency, Etiology, Symptomatology and Prognosis, Medical News, 1902, lxxxi, 481.
- Krumwiede, C., and Valentine, E. L.: (1) A Study of the Agglutination and Cultural Relationship of Members of the so-called Streptococcus-*viridans* Group, Jour. Infect. Dis., 1916, xix, 760.



- (2) A Bacteriological Study of an Epidemic of Septic Sore Throat, *Jour. Med. Research*, 1915, xxxiii, 231.
- Lamar, R. V.: Streptococcus Septicemia, *Jour. Exp. Med.*, 1909, xi, 152.
- Lamar, R. V., and Meltzer, S. J.: Experimental Bronchopneumonia by Intrabronchial Insufflation, *Jour. Exp. Med.*, 1912, xv, 133.
- Langowoy, N.: Beobachtungen über die Wirkung der Scharlach Streptokokken Vaccine, *Centralbl. f. Bakteriol.*, 1906, xlii, 362, 463.
- LeCount, E. R., and Jackson, L.: The Renal Changes in Rabbits Inoculated with Streptococci, *Jour. Infect. Dis.*, 1914, xv, 389.
- Leutscher, J. A.: Bacteriology of Epidemic Sore Throat, *Jour. Am. Med. Assn.*, 1912, lix, 868.
- Levy, R. L., and Alexander, H. L.: The Predisposition of Streptococcus Carriers to the Complications of Measles, *Jour. Am. Med. Assn.*, 1918, lxx, 1827.
- Lingelsheim, W. v.: Streptokokken, *Handbuch der Pathogenen Mikroorganismen* Kolle and Wassermann, Ed. 2, 1912, iv, 453.
- Longcope, W. T.: Streptococcus Mucosus (Howard) and its Relation to Micrococcus Lanceolatus, *Jour. Med. Research*, 1902, vii, 220.
- Lorey, A.: Bakteriologische Untersuchungen bei Masern, *Zeit. für Hygiene*, 1909, lxiii, 135.
- Lucke, B.: Postmortem Findings in Measles, Bronchopneumonia and Other Acute Infections, *Jour. Am. Med. Assn.*, 1918, lxx, 26, 2005.
- Lyall, H. W.: (1) On the Classification of the Streptococci, *Jour. Med. Research*, 1914, xxx, 487. (2) Observations on Hemolysin Produced by the Streptococci, *Jour. Med. Research*, 1914, xxx, 515.
- MacCallum, W. G., and Cole, R.: Pneumonia at a Base Hospital, *Jour. Am. Med. Assn.*, 1918, lxx, 1146.
- Marmorek, A.: (1) Le Streptocoque et le serum antistreptococcique, *Annales Pasteur*, 1895. (2) La Toxine Streptococcique, *Annales de l'Institut Pasteur*, 1902, xvi, 169.
- Marxer, A.: Weitere Untersuchungen zur Frage der Arteneinheit der Streptokokken, *Centralbl. f. Bakteriol.*, 1911, ix, 79.
- Mathers, G.: Different Types of Streptococci and Their Relation to Bovine Mastitis, *Jour. Infect. Dis.*, 1916, xix, 222.
- (2) The Streptococcus in an Acute Epidemic Respiratory Infection of Horses, *Jour. Infect. Dis.*, 1918, xxii, 74.
- (3) Some Bacteriologic Observations on Epidemic Poliomyelitis, *Jour. Am. Med. Assn.*, 1916, lxxvii, 1019.
- Mathers, G., and Howell, K.: Immune Reactions in Rabbits Injected with Micrococci from Acute Poliomyelitis, *Jour. Infect. Dis.*, 1917, xxi, 292.
- McLeod, J. W.: Criteria of Virulence Amongst Streptococci with Some Remarks on Streptococcal Leucocidin, *Jour. Path. and Bacteriol.*, 1915, xix, 392.
- Meakins, J. C.: Streptococcus Immunity, *Jour. Exper. Med.*, 1909, xi, 815.
- Menzer, A.: (1) Die Aetiologie des Acuten Gelenkrheumatismus, Berlin, 1902. (2) Das Antistreptokokken Serum und Seine Anwendung beim Menschen, München, med. Wehnschr., 1903, I, 1057-1125.
- Meyer, F.: Die Antistreptokokken Sera und ihre Klinische Anwendung, In *Handbuch der Serumtherapie*, I, 96, Wolff-Eisner Lehmanns, Munich, 1910.
- Moody, A. M.: Lesions in Rabbits Produced by Streptococci from Chronic Alveolar Abscesses, *Jour. Infect. Dis.*, 1916, xix, 515.
- Moore, J. J.: The Action of Vaccine and of Concentrated Antistreptococcus Serum in Experimental Streptococcal Arthritis, *Jour. Infect. Dis.*, 1914, xv, 215.
- Moser, P.: Ueber die Behandlung des Scharlach mit einem Scharlach Streptococceen Serum, *Wien. klin. Wehnschr.*, 1902, xv, 1053.
- Moser, P., and von Pirquet, C.: Zur Agglutination der Streptokokken, *Centralbl. f. Bakteriol.*, 1903, xxxiv, pp. 560, 714.
- Neufeld, F.: Ueber Immunität und Agglutination bei Streptokokken, *Zeit. für Hygiene*, 1903, xlv, 161.
- Neufeld, F., and Rimpau, W.: Ueber die Antikörper des Streptokokken und Pneumokokken Immunsersums, *Deutsch. med. Wehnschr.*, 1904, xxx, 1458.
- North, C. E., White, B., and Avery, O. T.: A Septic Sore Throat Epidemic in Cortland and Homer, N. Y., *Jour. Infect. Dis.*, 1914, xiv, 124.
- Nuzum, J. W., and Herzog, M.: Experimental Studies in the Etiology of Acute Epidemic Poliomyelitis, *Jour. Am. Med. Assn.*, 1916, lxxvii, 1204.
- Nuzum, J. W., and Willy, R. G.: Further Studies of an Antipoliomyelitis Serum, its Protective and Curative Properties in Experimental Poliomyelitis of Monkeys, *Jour. Infect. Dis.*, 1918, xxii, 258.
- Perkins, W. T., and Pay, G. O.: Streptococcus Pyogenes in Variola, *Jour. Med. Research*, 1904, x, 180.
- Pierce, R. W. C.: Epidemic Sore Throat and Suppurative Mammitis in Cows, *Brit. Med. Jour.*, 1903, ii, 1493.
- Poynton and Paine: Etiology of Rheumatic Fever, *Lancet*, London, 1900, pp. 800, 932.

- Quigley, W. J.: Observations on the Bacteriology of Chorea, *Jour. Infect. Dis.*, 1918, xxii, 198.
- Rosenow, E. C.: (1) Studies in Pneumonia and Pneumococcus Infections, *Jour. Infect. Dis.*, 1904, i, 280.
- (2) A Study of Streptococci from Milk and from Epidemic Sore Throat and the Effect of Milk on Streptococci, *Jour. Infect. Dis.*, 1912, xi, 339.
- (3) Transmutations with the Streptococcus Pneumococcus Group, *Jour. Infect. Dis.*, 1914, xiv, 1.
- (4) The Bacteriology of Appendicitis and its Production by Intravenous Injection of Streptococci and Colon Bacilli, *Jour. Infect. Dis.*, 1915, xvi, 240.
- (5) The Causation of Gastric and Duodenal Ulcer by Streptococci, *Jour. Infect. Dis.*, 1916, xix, 333.
- (6) Bacteriology of Cholecystitis and its Production by Injection of Streptococci, *Jour. Am. Med. Assn.*, 1914, lxiii, 1835.
- (7) Etiology and Experimental Production of Erythema Nodosum, *Jour. Infect. Dis.*, 1915, xvi, 367.
- (8) Iritis and Other Ocular Lesions on Intravenous Injection of Streptococci, *Jour. Infect. Dis.*, 1915, xvii, 403.
- (9) Elective Localization of Streptococci, *Jour. Am. Med. Assn.*, 1915, lxv, 1687.
- (10) The Newer Bacteriology of Various Infections as Determined by Special Methods, *Jour. Am. Med. Assn.*, 1914, lxiii, 903.
- (11) Report on the Treatment of Fifty-eight Cases of Epidemic Poliomyelitis with Immune Horse Serum, *Jour. Infect. Dis.*, 1918, xxii, 379.
- Rosenow, E. C., and Dunlap, S. I.: An Epidemic of Appendicitis and Parotitis Probably Due to Streptococci Contained in Dairy Products, *Jour. Infect. Dis.*, 1916, xviii, 383.
- Rosenow, E. C., and Gray, H.: Agglutination of the Pleomorphic Streptococcus Isolated from Epidemic Poliomyelitis by Immune Serum, *Jour. Infect. Dis.*, 1918, xxii, 345.
- Rosenow, E. C., and Moon, V. H.: On an Epidemic of Sore Throat and the Virulence of Streptococci Isolated from the Milk, *Jour. Infect. Dis.*, 1915, xvii, 69.
- Rosenow, E. C., and Towne, E. B.: Bacteriological Observations in Experimental Poliomyelitis of Monkeys, *Jour. Med. Research*, 1917, xxxvi, 175.
- Rosenow, E. C., and Wheeler, C. W.: The Etiology of Epidemic Poliomyelitis, *Jour. Infect. Dis.*, 1918, xxii, 281.
- Rothschild, W., and Thalheimer, M. A.: Arthritis Produced by Streptococcus Mitis, *Jour. Exper. Med.*, 1914, xix, 444.
- Ruediger, G. F.: (1) The Streptococci from Scarlatinal and Normal Throats and from Other Sources, *Jour. Infect. Dis.*, 1906, iii, 755.
- (2) A Study of the Nature of Streptolysin, *Jour. Infect. Dis.*, 1907, iv, 277.
- (3) The Cause of Green Coloration of Bacterial Colonies in Blood-agar Plates, *Jour. Infect. Dis.*, 1906, iii, 663.
- (4) The Production and Nature of Streptocolsin, *Jour. Am. Med. Assn.*, 1903 (October 17, 1903).
- (5) A Study of Thirty-five Strains of Streptococci Isolated from Milk, *Soc. Am. Bacteriol. Science*, 1912, xxxv, 223.
- (6) The Mechanism of Streptococcus Infection, *Jour. Am. Med. Assn.*, 1905 (Jan. 21, 1905).
- (7) Further Studies on Streptococcus Infections, *Jour. Infect. Dis.*, 1906, iii, 156.
- (8) The Effects on Streptococci of Sera of Cold-blooded Animals, *Jour. Infect. Dis.*, 1904, i, 107.
- Savage, W. G.: Milk and the Public Health, MacMillan, 1912, p. 1107.
- Schenk, F.: Experimentelle zur Frage der Streptokokkenimmunität, *Zeit. für Hygiene*, 1914, lxxvi, 307.
- Schloss, O. N., and Foster, N. B.: Experimental Streptococcic Arthritis in Monkeys, *Jour. Med. Research*, 1913, xxix, 9.
- Schottmüller: Die Artunterscheidung der für den Menschen Pathogenen Streptokokken durch Blut Agar, *München. med. Wchnschr.*, 1903, i, 849.
- Schutz: Die Ursache der Brustseuche der Pferde, *Virchow's Archiv*, 1887, pp. 107, 356.
- Schwoner, J.: Streptokokken serum, *Kraus und Levaditi's Handbuch der Immunitätsforschung*, ii, 481.
- Simon, F. B.: (1) Ueber spezifische Absorption Schützender Antikörper aus Streptokokken immunserum, *Cent. für Bakt.*, 1912, lxv, 206.
- (2) Experimentelle Untersuchungen über das monogene Streptokokken immunserum, *Cent. für Bakt.*, I Abt. Orig., 1907, xlv, 563-683.
- Simonds, J. P.: The Effect of the Injection of Killed Streptococci on the Streptococcosis Index of Normal Rabbits, *Jour. Infect. Dis.*, 1907, iv, 595.
- Smillie, W. G.: The Beta Hemolytic Streptococci, *Jour. Infect. Dis.*, 1917, xx, 45.
- Smith, T., and Brown, J. H.: A Study of Streptococci Isolated from Certain Presumably Milk-borne Epidemics of Tonsillitis Occurring in Massachusetts in 1913 and 1914, *Jour. Med. Research*, 1914-15, xxxi, 455.

- Stokes, W. R., and Hatchel, F. W.: Bacteriological Study of the Outbreak in Baltimore, Md. Reprint from Public Health Reports No. 103, Government Printing Office, 1912.
- Stowell, E. C., Hilliard, C. M., and Schlesinger, M. J.: A Statistical Study of the Streptococci from Milk and from Human Throat, *Jour. Infect. Dis.*, 1913, xii, 144.
- Swift and Kinsella: Bacteriologic Studies in Acute Rheumatic Fever, *Arch. Int. Med.*, 1917, xix, 381.
- Swift, H. T., and Thro, W. C.: A Study of Streptococci with the Complement Fixation and Conglutination Reactions, *Arch. Int. Med.*, 1911, vii, 24.
- Tavel: Ueber das Polyvalente Streptokokken serum, etc., *Centralbl. f. Bakteriöl.*, xxxiii, 1903, 212.
- Tchitchkine, A.: De l'action du streptocoque et de la lysine introduite par voie buccale et, *Annales Pasteur*, 1906, xx, 499.
- Thro, W. C.: (1) Experiments on the Variability of the Fermentative Reactions of Bacteria, Especially the Streptococci, *Jour. Infect. Dis.*, 1914, xv, 234.  
(2) Further Experiments on the Variability of the Fermentative Reaction of Bacteria, Especially the Streptococci, *Jour. Infect. Dis.*, 1915, xvii, 227.
- Thursfield: Report on an Enquiry into the Causes of Death in Measles, Report of the Medical Officer 1912-13, Great Britain, His Majesty's Stationary Office, Darling and Son, London, 1914.
- Tsen, E. T. H.: On the Isolation of Streptococci from Rabbits, *Proc. Soc. Exper. Biol. and Med.*, 1917, xiv, 112.
- Tunncliffe, R.: (1) The Streptococco Opsonic Index in Scarlatina, *Jour. Infect. Dis.*, 1907, vi, 304.  
(2) The Cultivation of a Micrococcus from Blood in Preeruptive Stages of Measles, *Jour. Am. Med. Assn.*, 1917, lxvii, 1028.  
(3) Observations on the Bacteriology and Immune Reactions of Rubcola (measles) and Rubella (German measles), *Jour. Infect. Dis.*, 1918, xxii, 462.
- Van de Velde, H.: De la nécessité une serum antistreptococcique polyvalent. *Archives de med. experimentale et d'anatomie pathologique*, 1897, ix, 835.
- Weaver, G. H.: (1) Agglutination of Streptococci, Especially those Cultivated from Cases of Scarlatina, by Human Sera, *Jour. Infect. Dis.*, 1904, i, 91.  
(2) The Treatment of Scarlet Fever with Immune Human Serum, *Jour. Infect. Dis.*, 1918, xxii, 211.  
(3) The Effects of Injections of Killed Streptococci, *Tr. Assn. Am. Phys.*, 1910.  
(4) Antistreptococcus Serum, Forchheimer's Therapeutics of International Diseases, v, 652, Appleton, 1914.
- Weaver, G. H., and Tunncliffe, R.: (1) A Study of the Action of Antistreptococcus Serum in Streptococci Infections in Man, *Jour. Infect. Dis.*, 1912, x, 321.  
(2) Further Study of Antistreptococcus Serum, *Jour. Infect. Dis.*, 1911, ix, 130.
- Weil, E.: Ueber die Wirkungsweise des Streptokokkenimmenserum, *Zeit. für Hyg.*, 1913, lxxv, 245.
- Westphall, Wassermann and Malkoff: Ueber den infectiösen Charakter und Zusammenhang von acuten Gelenkrheumatismus und Chorea, *Berl. klin. Wchnschr.*, 1899, xxxvi, 638.
- Winslow, C-E. A.: An Outbreak of Tonsillitis, or Septic Sore Throat in Eastern Massachusetts and its Relation to an Infected Milk Supply, *Jour. Infect. Dis.*, 1912, x, 73.
- Winslow, C-E. A., and Hubbard, L. W.: Epidemiology and Symptomatology of an Outbreak of Septic Sore Throat in Westchester County, New York, *Jour. Infect. Dis.*, 1916, xviii, 106.
- Winslow, C-E. A., and Palmer, G. T.: A Comparative Study of Intestinal Streptococci from the Horse, the Cow and Man, *Jour. Infect. Dis.*, 1910, vii, 1.
- Winternitz, M. C., and Hirschfelder, M. D.: Studies Upon Experimental Pneumonia in Rabbits, *Jour. Exp. Med.*, 1913, xvii, 657.
- Wlaskjewski: Ueber Streptokokken Agglutination, *Cent. für Bakt. I Abt. Referate*, 1903, xxxiii, 464.
- Wollstein, M., and Meltzer, S. J.: (1) Experimental Bronchopneumonia by Intrabronchial Insufflation, *Jour. Exper. Med.*, 1912, xvi, 126.  
(2) Pneumonic Lesions Produced by Streptococcus, *Jour. Exper. Med.*, 1913, xviii, 548.
- Wright, A. E.: Studies in Immunization, Constable, London, 1909.
- Zelenski, T.: Zur Agglutination der Streptokokken, *Wien. klin. Wchnschr.*, 1904, xvii, 406.
- Zybell, F.: Das Empyem im Säuglingsalter, *Ergebnisse der inneren Medizin und Kinderheilkunde*, 1913, ii, 611, Springer, Berlin.

## STUDY OF THE LEUCOCYTES IN AN EPIDEMIC OF INFLUENZA

BY ROY P. FORBES, CAPT. M.R.C., AND HELEN A. SNYDER, A.N.C.

**D**URING the month of April, 1918, a highly contagious, but comparatively mild infection of the respiratory tract was epidemic at Camp Hancock. Several thousand men in the command were infected, but relatively few were ill enough to be sent to the Base Hospital. The only fatal case occurred early in the epidemic, and it was the observations made in this case which prompted the further study of the blood picture.

*Report of case:* Sergeant G. L. P. was admitted to the Base Hospital on April 7, 1918, complaining of a cough of four days' duration and severe headache. The positive physical findings included only severe conjunctivitis and a few rales at the left base. The temperature on admission was 101, pulse 94, respirations 22. The day following admission signs of pulmonary consolidation were found. The respirations had increased to 40. The leucocyte count was 4300. A blood culture was taken on April 9, which showed in twenty-four hours a heavy growth of small nonmotile, gram-negative, bacilli, *B. influenzae*. The leucocyte counts made on this date at five hour intervals were respectively 3400, 2200, and 2300. A second blood culture taken on April 10 also showed a pure culture of the same organism. The leucocyte count had risen to 5600. Delirium and prostration were marked and death ensued on April 11. At necropsy a confluent bronchopneumonia was found. Both lungs were quite heavy and almost completely infiltrated. There was no pleural effusion and the other viscera showed nothing remarkable. *B. influenzae* was recovered from cultures taken from the lung and spleen. One-half cubic centimeter of a twenty-four hour culture of the organism injected into the peritoneum of a young rat failed to cause death.

Early in the epidemic it was noted that leucopenia or at least absence of hyperleucocytosis, was a constant factor, and the leucocyte count was frequently of aid in differential diagnosis. However, the clinical picture of the disease usually made diagnosis easy: Nearly every patient gave as the initial symptoms backache, headache and slight cough or sore throat. Conjunctivitis and a marked injection of the soft palate was noted in 90 per cent of the cases. In addition, a slight or moderate general adenopathy was often noted. The face was flushed and in a few cases the skin of the thorax presented a mild erythema. In three cases a provisional diagnosis of scarlet fever was made until the blood count showed a leucopenia. Another case diagnosed influenza, showing a leucocytosis of 18,000, was isolated and promptly developed a typical scarlet rash.

Positive bacterial findings are necessary in establishing the diagnosis of influenza. Nasopharynx cultures on plates showed a very small, gram-negative bacillus, morphologically *B. influenzae* in seventeen of thirty-three cases, but the identity of the organisms was unfortunately not established by subcultures. In ten cases in which sputum examinations were made, *B. influenzae*



was found in only four, Streptococci were present in six. Blood cultures on twenty consecutive nonfatal cases, only one of which was complicated by bronchopneumonia, were all negative. No conclusions can be drawn from our bacteriological findings. In this connection it is interesting to note that Moody and Capps<sup>1</sup> found influenza bacilli in only two out of thirty-one cases studied. These workers found that a streptococcus was the most constant organism in the 1916 epidemic. Mathers<sup>2</sup> working in the same epidemic, found influenza bacilli in only one of sixty-one cases. He concluded that the hemolytic streptococcus was the causative organism in the epidemic. The literature contains very little data concerning the leucocytes in influenza. Simon states that absence of hyperleucocytosis, together with relative lymphocytosis, are essential features of the disease. In Cabot's series of three hundred and nine cases, he found the leucocytes over 10,000 in one hundred and thirty-five cases, or about 42 per cent of the series. In our series the leucocytes exceeded 10,000 in only 22 per cent of the cases and never exceeded 15,000.

TABLE I

1. Number of cases studied .....	50
2. Number of white blood counts.....	202
3. Total number of cases with one or more leucocyte counts below 5,000....	13
4. Total number of cases with one or more leucocyte counts above 10,000....	11
5. Average differential count on 16 cases	
a. 1st to 3rd day:	
Polymorphonuclears .....	49.65
Total large and small mononuclears and transitionals.....	40.72
Eosinophiles .....	.63
Basophiles .....	.00
b. 4th to 6th day:	
Polymorphonuclears .....	57.65
Total large and small mononuclears and transitionals.....	41.72
Eosinophiles .....	.60
Basophiles .....	.03

TABLE II

DAY OF DISEASE	SUMMARY OF 202 COUNTS IN 50 CASES		
	NUMBER OF CASES WITH LEUCOCYTES ABOVE 5,000	NUMBER OF CASES WITH LEUCOCYTES ABOVE 10,000	AVERAGE OF LEUCOCYTE COUNTS
1	6	1	6,166
2	1	2	5,378
3	2	2	7,522
4	2	4	8,158
5	0	2	8,059
6	2*	4	7,885

\*Both of these cases had a complicating bronchopneumonia.

## COMMENT

Some of the cases were not under observation on the first or second day of the disease and some were discharged before the sixth day, so that six consecutive leucocyte counts were not always obtainable. However, the averages of two hundred and two counts are considered to be fair estimates. In a few cases not here recorded in which differential counts were made from ten days to three weeks after the illness the relative lymphocytosis had not entirely disap-

peared. Apparently a complicating bronchopneumonia decreases rather than increases the lymphocytes, because the lowest counts were noted in the two cases which developed pneumonia. There was a considerable variation in the degree of lymphocytosis. Ten of the thirty-two preparations gave a total mononuclear count above 50 per cent. The increase in the mononuclear elements was nearly all confined to the lymphocytes.

#### CONCLUSIONS

1. Absence of hyperleucocytosis or actual leucopenia, and relative lymphocytosis are characteristic of influenza.
2. The leucocyte count may be of value in the early diagnosis of influenza and for differentiating it from a beginning scarlet rash.
3. Influenza bacilli are not found in the blood stream in the ordinary, mild cases, but may be found in a very severe or fatal case.

#### BIBLIOGRAPHY

- <sup>1</sup>Moody and Capps: Jour. A. M. A., lxvi, No. 22, p. 1696.  
<sup>2</sup>Mathers: Jour. Infect. Dis., xxi, No. 1, p. 18.  
<sup>3</sup>Capps and Moody: Jour. A. M. A., lxvii, No. 19, pp. 1349-50.

# LABORATORY METHODS

---

## INTRODUCTORY EXERCISES IN EXPERIMENTAL PATHOLOGY

---

By WILFRED H. MANWARING, M.D., STANFORD UNIVERSITY, CAL.

---

THE required second-year course in General Pathology in Stanford University Medical School is divided into two parts. The first part, "General Pathology A," extends throughout a university term of eleven weeks, and covers the ground of circulatory disturbances, degenerations, pigmentations, acute inflammations and regenerations. This part is given by the Department of Bacteriology and Experimental Pathology, of Leland Stanford Junior University, at Stanford University, Cal.

The second part, "General Pathology B," covers the ground of infectious granulomata and tumors, and is given by the Division of Pathology (Doctor Ophüls), of Stanford Medical School at San Francisco. The second-year course in General Pathology is succeeded by a course in Special Pathology, given by Doctor Ophüls, and extending throughout the third medical year.

The introductory course, "General Pathology A," is given from the experimental point of view. The course consists largely of aseptic operations on animals, with a gross and microscopic study of the experimental material thus obtained. The operative material is supplemented by material from human autopsies illustrating the same changes.

A weekly program of General Pathology A is given in Schedule I. A detailed outline of the experimental part of the course is given in Schedule II. The scheduled student operations are supplemented by class demonstrations illustrating more difficult processes.

### SCHEDULE I. WEEKLY PROGRAM. GENERAL PATHOLOGY A

Lecture Monday, Wednesday and Friday, 8 o'clock, Pathologic-histologic Laboratory	
Monday, Wednesday and Friday, 9 to 11.....	6 units
Preparation of drawings (optional) .....	2 units
Aseptic surgical operations, Tuesday, Thursday and Friday, 8 to 11.....	2 units
Pathologic-histologic technic (elective) .....	2 units

1st week—Wed.: Scope of General Pathology. Thurs.: Histologic Technic, Aseptic Surgical Technic. Fri.: Operations.

2nd week—Mon.: Congestion, Stasis. Wed., Fri.: Cohnheim's Frog Experiment, Simple Inflammations, Exudates (begun). Tues., Thurs.: Operations.

3rd week—Mon.: Hemorrhage. Wed.: Thrombosis. Tues., Thurs., Fri.: Operations, Autopsies.

4th week—Mon.: Embolism. Wed.: Local Anemias, Infarcts. Tues., Thurs., Fri.: Operations, Autopsies.

5th week—Mon.: Necrosis. Wed.: Atrophy. Tues., Thurs., Fri.: Operations, Autopsies.

- 6th week—Mon.: Disturbances of Protein Metabolism. Wed.: Hyaline, Amyloid. Fri.: Disturbances of Fat Metabolism, Fat Necrosis. Tues., Thurs.: Operations, Autopsies.
- 7th week—Mon.: Disturbances of Carbohydrate and Mineral Metabolism Calcification. Wed.: Disturbances of Pigment Metabolism, Hematogenous Pigmentation. Fri.: Icterus, Exogenous Pigments, Non-pigmented Waste Products. Tues., Thurs.: Operations, Autopsies.
- 8th week—Mon.: Concretions, Obstructions, Retentions, Cysts. Wed.: Edema, Transudates. Fri.: Acute Inflammations (continued). Tues., Thurs.: Operations, Autopsies.
- 9th week—Mon.: Experimental Tuberculosis. Wed.: Organizations, Regenerations, Wound Healing, Transplantations. Fri.: Physiologic Compensations, Hypertrophy. Tues., Thurs.: Autopsies.
- 10th week—Mon., Tues., Wed., Thurs., and Fri.: Practical Examination. (Identification of unknowns, special technic.)
- 11th week—Written examination.

## SCHEDULE II. ASEPTIC SURGICAL OPERATIONS. GENERAL PATHOLOGY A

Tuesday, Thursday (Friday), 8 to 11 o'clock.....2 units

The following operations are to be performed by each double surgical group of eight students. Unsuccessful operations are to be repeated during the optional operative period (Friday). The operations are to be made with strictest aseptic and antiseptic precautions, under either or morphine-ether anesthesia. The animals are to be kept in specially heated rooms for at least 48 hours after the operations.

In the routine autopsy, the animal is first exsanguinated from the carotids, under chloroform or ether anesthesia, care being taken not to cut the trachea. In all cases an examination is to be made of both abdominal and thoracic viscera. The accessory findings are often more important than the specific lesion under consideration. Smears are to be made from all exudates and transudates. Bacteriologic and histologic examinations as directed.

### 1st Week

FRIDAY.—1. Ligate left renal vein, 2 rabbits. Aspirate sample of urine and remove both kidneys of each animal 2 hours later, ligating remaining blood vessels and ureters to prevent escape of blood. Preserve excised organs in Kaiserling's solution. After hardening, split each organ lengthwise. Make colored sketch of the cut surfaces. Study histologic material from previous operations.

### 2nd Week

TUESDAY.—2. Ligate left renal artery, 4 rabbits. Autopsies 4, 12, 30, and 60 days later.

THURSDAY.—3. Inject 10 c.c. 5% aleuronat (in 2% starch paste) into right pleural cavity, 2 rabbits. Exsanguinate 18 to 24 hours later, open abdomen, aspirate pleural fluid through diaphragm. Make numerous smears, test coagulability of one sample, examine one sample in unstained condition subsequently running dilute methylene blue (1:4 in NaCl solution) under the cover glass. Add to one sample an equal volume of a strong suspension of *S. pneumoniae*, incubate the mixture, make smears at the end of 15, 30, and 60 minutes (Gram's stain).

4. Ligate branch, left renal artery, 4 rabbits. Make sketches showing immediate physiologic reactions. Autopsies with additional sketches 4, 12, 30, and 60 days later.

### 3rd Week

TUESDAY.—5. Isolate samples of circulating blood between carefully tied ligatures, jugular vein, 2 rabbits, crushing one sample between forceps. Incise samples 1 hour later. Save animals for Exp. 6.

6. Inject 20 c.c. defibrinated blood (filter through glass wool), peritoneal cavity, rabbit; 10 c.c. freshly drawn uncoagulated blood, pleural cavity, rabbit. Autopsy 2 days later.

7. Isolate portion of jugular vein between clamps, 2 dogs. Aspirate blood through a ligated collateral, wash out isolated portion with distilled water, inject 2 per cent  $\text{AgNO}_3$ .



and immediately wash out with NaCl solution, ligate puncture wound, release clamps. Autopsies 1 and 2 days later.

THURSDAY.—8. Ligate left ureter, rabbit, cat, puppy. Autopsies 4 to 6 weeks later.

9. Nephrectomy, 2 adult dogs. Weigh and measure excised organs, preserve in Kaiserling's solution. Heavy meat diet. Autopsies 6 to 8 weeks later.

#### 4th Week

TUESDAY.—10. Ligate adjacent branches, mesenteric artery; elastic ligature about loop of intestine; experimental intussusception; 2 dogs. Autopsy 2 to 3 days later.

11. Ligate large branch of renal artery, 2 dogs. Autopsies 1 week and 4 to 6 weeks later.

12. Ligate vena cava, immediately below liver, 2 rabbits. Autopsies 4 to 6 weeks later.

THURSDAY.—13. End-to-end anastomosis, intestine, 2 cats. Autopsies 1 to 2 weeks and 4 to 8 weeks later.

14. Lateral anastomosis, intestine, 2 dogs. Autopsies 1 week and 4 to 6 weeks later.

#### 5th Week

TUESDAY.—15. Experimental fracture, ribs, 2 rabbits. Autopsies 2 weeks and 4 to 6 weeks later.

16. Bone transplantation, femur, 2 dogs. Autopsies 2 and 4 to 6 weeks later.

THURSDAY.—17. Ligate common bile duct, 2 dogs. Autopsies 10 to 12 days later. Examine urine, feces.

18. Heat injury, femur, 2 rabbits. Autopsies 4 to 6 weeks later.

#### 6th Week

THURSDAY.—19. Inject 2 small rabbits intravenously, at 2 to 3 day intervals, 3 to 5 doses, 5 c.c. 10 per cent India ink (in NaCl solution). Autopsy 3 days after final dose.

20. Introduce rubber cylinder 1 cm.  $\times$  4 mm. in diameter, into large bronchus, 2 dogs. Autopsy 2 to 3 weeks later.

#### 7th Week

THURSDAY.—21. Three per cent  $\text{AgNO}_3$  injury, intestine, liver, 2 cats. Autopsies 1 and 2 weeks later.

22. Temporary ligature, renal artery, 4 rabbits. Remove ligatures at end of 2 hours, noting immediate physiologic reactions. Autopsies 1, 2, 4, and 10 days later.

#### 8th Week

TUESDAY.—23. 0.1 c.c. 24 hr. broth *S. aureus*, intravenously, 2 rabbits; 1 c.c. 24 hr. broth *S. aureus*, intrapleurally, 2 rabbits; 1 c.c. 24 hr. broth *S. aureus* subcutaneously, 2 rabbits. Autopsies 2 to 4 days later.

24. Introduce 10 to 20 c.c. 24 hr. broth *S. pneumoniae*, by intrabronchial insufflation, into pulmonary alveoli, 2 dogs. Autopsies, with bacteriologic study; 1 to 4 days later.

#### 9th Week

25. Tuberculosis injections, guinea pigs, rabbits, dogs, 2nd to 5th week. Autopsies 9th week.

### SUPPLEMENTARY OPERATIONS

During the latter half of the term an opportunity is given each surgical group to do two or more voluntary operations. The following are among the operations recommended:

1. End-to-side anastomosis, ureter, 2 dogs. Autopsies 2 and 4 weeks later.
2. End-to-end anastomosis, jugular vein, 2 dogs. Autopsies 24 hours and 2 weeks later.
3. Hemisection, spinal cord, 2 dogs. Autopsies 1 and 2 weeks later.
4. Excise portion of cerebral cortex, 2 dogs. Autopsies 1 and 2 weeks later.
5. End-to-end anastomosis, sciatic nerve, 2 dogs. Autopsies 2 and 4 weeks later.

6. Experimental fracture, humerus, 2 dogs. Use steel bone plate. Autopsies 2 and 4 weeks later.
7. Ligation of pancreatic duct, 2 dogs. Autopsies 2 and 4 weeks later.
8. Experimental pyloric stenosis with gastroenterostomy, 2 dogs. Autopsies 2 and 4 weeks later.
9. Bilateral adrenalectomy, 1 dog; bilateral adrenalectomy with transplantation of excised adrenals into kidney, 1 dog. Autopsy of living animal 2 to 4 weeks later.
10. Eck fistula, 2 young dogs. Autopsies 2 to 4 weeks later.

The equipment for the experimental work consists of a series of small operating rooms, with adjacent autopsy room, sterilizing room, preparation room, animal rooms, dog kennels, etc. The cost of the experimental work is about \$10 a student, the students working in groups of four. The groups are divided into subgroups of two for operations on smaller animals. It is our custom to charge an extra fee for the experimental work, sufficient to cover half the cost of animals, feed, and other materials used.

The experimental course has been favorably received by our medical students. The course gives a more vivid conception of elementary pathologic processes than that obtained from the usual introductory study of human autopsy material. It is our plan to expand the course next year to include certain introductory exercises in pathologic physiology.

No objection to this work has been made by local anti-vivisectionists.\* In fact, one gains the impression that the general introduction of such operative courses in our medical schools would be an efficient way to meet the present anti-vivisection movement.

---

\*See this JOURNAL, 1917, ii, 573.

# *The Journal of Laboratory and Clinical Medicine*

VOL. III.

SEPTEMBER, 1918

No. 12

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.  
Ann Arbor, Mich.

## ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D.	- - -	ST. LOUIS
HANS ZINSSER, M.D.	- - -	NEW YORK
PAUL G. WOOLLEY, M.D.	- - -	CINCINNATI
FREDERICK P. GAY, M.D.	- - -	BERKELEY, CAL.
J. J. R. MACLEOD, M.B.	- - -	TORONTO
ROY G. PEARCE, M.D.	- - -	CLEVELAND
ROGER S. MORRIS, M.D.	- - -	CINCINNATI
GERALD B. WEBB, M.D.	- - -	COLORADO SPRINGS
E. E. SOUTHARD, M.D.	- - -	BOSTON

Contents of this Journal Copyright, 1918, by The C. V. Mosby Company—All Rights Reserved  
Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

## EDITORIALS

### *Pulmonary Tuberculosis*

THROUGH the centuries the bacillus of tuberculosis has established a mode of access to and egress from the human lungs.

From the species point of view—the desire for a “place in the sun”—the tubercle bacillus must found new colonies from which again it can prepare new campaigns.

This bacillus, as Theobald Smith points out, is “tuned up” for the adult lung and in consequence it is in adult life that we see best the achievements of its militarism! Destruction of the colonized host would seem an accident in the life history of this parasite, the bacillus desiring domination by its “kultur” and not death.

Light is being shed on the manner in which the aims of the tubercle bacillus are achieved.

The average death rate today from pulmonary tuberculosis is approaching fifty years and as many are victims between sixty-five and seventy-five years as between twenty and twenty-five years.

Dating from childhood infection it would then appear that the bacilli may operate at times for fifty years before the host is destroyed.

Opie<sup>1</sup> has indicated that the lungs of almost every adult contain foci of tubercle which are characteristic of the infection of childhood. These focal infections originate usually between the ages of ten and eighteen, and in adults are almost always found to be calcified and healed. Between eighteen and thirty some 85 per cent of autopsies have revealed focal tubercle.

It is argued that apical tuberculosis and fatal pulmonary tuberculosis are common in later life when focal childhood infections are considered healed.

Autopsy studies are related to show that the incidence of encapsulated and of healed lesions in the apices increases with increasing age but that there is no similar increase of active tuberculosis.

The situation is compared by Opie to that of the early guinea pig experiments of Koch in which lymph nodes adjacent to the site of a second inoculation of a tuberculous animal are found to be free from caseation.

Many of the apical lesions are found to be fresh and caseous but the regional lymph nodes are not caseated.

Additional support is given by reference to the lesions in monkeys and in miliary tuberculosis when caseation of lymph nodes is found similar to those adjacent to the site of Koch's initial injection of bacilli, but no early healed infection is discovered.

Focal lesions of childhood also can be found in one lung whereas the apical lesion may be in the opposite lung.

Opie suggests that such a relationship affords no support to the view that tuberculous lesions may be transmitted to the apex by the lymphatics. His arguments and demonstrations suggest that the apical and fatal forms of tuberculosis of the adult may possibly be a secondary infection from without. They fail to reveal, however, why these infections should almost invariably be apical. The clinical and pathologic teachings current today are<sup>2</sup> exceedingly well presented in an article by Bushnell.

From a hilus infection, the origin of which is not discussed, the tubercle bacillus is described as travelling by the peribronchial lymph spaces, in a direction opposite to that of the normal lymph flow, to a region in the upper lobes where the lymph motion is most sluggish.

As Opie grants it is conceivable that such progress might be aided by a reversal of the lymph current and such a reversal might very well occur as a result of a block at the hilus.

The tubercle bacillus travelling by these spaces to the parenchyma is resisted by the tissue cells and the type of peribronchial tuberculosis results. Caseation through the bronchus may occur although peribronchial tuberculosis is more often of the "closed" type as is evidenced clinically by the less frequent finding of the bacilli in the sputum and the rarer occurrence of hemorrhage in such cases (Heise and Sampson<sup>3</sup>).

Having reached the alveoli either encapsulation, caseation or cavity formation may proceed. By the latter the bacilli have achieved par excellence



their destiny as now by myriads they may be expectorated and thence find new colonies.

Occasionally we observe an aberrant method of escape from the chest. Tuberculosis of the sternum and of the ribs has in recent years been traced to prior pleural or mediastinum sources (Robinson<sup>4</sup>).

Another example of bacteria using the lymph channels to reach the periphery is pictured in the report of McCallum<sup>5</sup> on the pathology of bronchopneumonia. It is demonstrated that the streptococcus hemolyticus reaches the pleura by the lymph spaces in the walls of the bronchi, blood vessels and septa.

With or possibly without the formation of peribronchial tubercles it is conceivable then that tubercle bacilli can reach the subpleural lymph trunks or network, and latent bacilli in this region could originate the tuberculous pleurisies.

It is not uncommon, as depicted so beautifully by Letulle<sup>6</sup> to find on microscopic examination subpleural lines of tubercle.

Letulle's exquisite plates also show well the spread of the peribronchial tubercles to the parenchyma and also to the interior of the bronchus by destruction of the wall.

The analogy with the streptococcus hemolyticus, the careful study of pathologic specimens, combined with the knowledge gained by x-ray interpretations, suggest therefore that the conception of pulmonary tuberculosis so well presented by Bushnell will probably prove correct. More minute pathologic study may trace the fatal apical tuberculosis of adults to the focal infections of childhood.

#### BIBLIOGRAPHY

<sup>1</sup>Opie: Jour. Exper. Med., August, 1917; Ibid., 1917, xxv, 855.

<sup>2</sup>Bushnell: Military Surgeon, April, 1918.

<sup>3</sup>Heise and Sampson: Am. Rev. Tuberculosis, Feb., 1918.

<sup>4</sup>Robinson: Tran. National Tuberculosis Assn., 1917.

<sup>5</sup>McCallum: Jour. Am. Med. Assn., April 20, 1918.

<sup>6</sup>Letulle: Tuberculose Pleuro-pulmonaire, Paris, 1916.

—G. B. H.

### *Cigarette Smokers and Pulmonary Tuberculosis*

IT was observed during the examination of certain military commands for tuberculosis that some 30 per cent of the men discharged on account of this disease were men who did not inhale tobacco smoke. Among those who inhaled the smoke from cigarettes and who had no disease Webb<sup>1</sup> recorded evidences of indeterminate or sibilant rales suggestive of bronchitis.

Krause<sup>2</sup> in an interesting discussion of this paper dwells on the question of tissue irritation and reaction and its relation to infection. He suggests that an area of inflammation is probably not a *locus minoris resistentiae* but rather a

<sup>1</sup>Webb, G. B.: Mil. Surgeon, April, 1918.

<sup>2</sup>Krause, A. K.: Am. Rev. Tuberculosis, April, 1918.

point of heightened resistance to fresh infection. A tissue in a state of reaction like a varicose ulcer is not usually the seat for an erysipelas, but rather a skin in normal condition would appear more vulnerable.

There is, however, an additional immunological principle to be considered and that is the matter of lymph flow and stasis.

Lymph stasis favors as we know the deposit of tubercle.

In the examinations above referred to it was noted that more moisture could be detected in the normal chests of those who were excessive cigarette smokers (fifteen to forty cigarettes a day) than in the moderate inhalers.

Webb pointed out the presence at times of coarse mucous rales in the lungs of excessive inhalers, and also the quiet vesicular breathing, a further evidence of wet bronchi.

It is suggestive therefore that through the slight persistent irritation of the inhalation of cigarette smoke a slight tissue inflammation and reaction is set up and an increased lymph flow is stimulated.

We are today considering the probability that tubercle spreads from the hilus through and along the peribronchial lymph channels to reach the periphery, and that on account of the sluggish lymph movement in the apices here are found the classical processes.

It is quite logical to consider therefore that the above discussed processes of tissue irritation and increased lymph flow might aid in resisting the projects of the tubercle bacillus.

Could such a thought be considered a therapeutic "lead" what a prospective joyful type of treatment to many!

That so marked a proportion of those with pulmonary tuberculosis exists among the soldiers who do not inhale tobacco smoke these days, suggests as Osler remarked in another connection "Is there something wrong with the blastoderm?"

O. M. Gilbert's observation that cigar and pipe smokers would appear to suffer more from the absorption of nicotine than do the cigarette devotees, must also be kept in mind when considering the effects of tobacco. It might be shown that such absorption could lend aid to the development of tubercle, and it will require much study and many figures to determine such a question.

—G. B. W.

---

## ERRATA

In the article "Tolerance and Immunity," by John L. Marchand, M.D., Prinsapolka, Nicaragua, C. A., in the July, 1918, issue of the JOURNAL, read as follows:

Page 585, first word, second line, read *staphylococci* instead of streptococci.

Page 589, ninth word, twelfth line, read *staphyloproctin* instead of streptoprotein.

# INDEX TO VOLUME III

## AUTHORS INDEX

In this index following the author's name the full title of the subject is given as it appears in the Journal. Editorials are also included in this list and are indicated by (E).

### A

- ASKENSTEDT, FRITZ C. A simple test for glycuronates in the urine, 300  
 AVERY, OSWALD T. (See Holman, Avery, Kinsella, Brown), 618

### B

- BARNEY, E. L. A discussion of the lipoids concerned in growth with clinical observations on the action of tethelin, 480  
 BARRON, MOSES. A highly differentiating polychromatic toluidin-blue stain, 432  
 BARTLETT, C. J., and O'SHANSKY, A. L. A modified Wassermann technic based upon the rapid fixation of complement present in human serum, 118  
 BEAVER, DONALD C. (See Ward and Beaver), 348  
 BLUMBERG, ALFRED. Studies on immunity with special reference to complement fixation, 397  
 BREUER, MILES J. Aids to laboratory efficiency, 241  
 BRONFENBRENNER, J. On the complement-fixation test in tuberculosis with Besredka's antigen, 51  
 BROWN, J. HOWARD. (See Holman, Avery, Kinsella, and Brown, 618)  
 BRUCE, W. J. (See Walker and Bruce), 434

### C

- COHEN, M. B. An experimental study of root-filled teeth: Preliminary report, 202  
 COURTNEY, R. H. (See Haskell and Courtney), 110  
 CULVER, HARRY. Antibodies in gonococcal arthritis after the intravenous injection of specific and nonspecific protein, 12  
 — The gonococcal action of protein silver solution in vitro, 487

### D

- DAVIS, LEWIS. An investigation of the chemical composition and biologic availability of peptone, 75  
 — Studies on diphtheria toxin, 358  
 DOWNS, ARDREY W., and HAYS, GEORGE. Two suggestions of apparatus for the teaching laboratory, 553

### E

- ECKFORD, W. H. On the development of a method for early diagnosis of tuberculosis by the use of the x-rayed guinea pig, 175  
 EDITORIAL. Volunteer medical service corps, 381

### F

- FANTUS, BERNARD. Tungstates of alkaloids—an experimental pharmacologic study, 179  
 FERRY, N. S., and NOBLE, ARLYLE. Peptone-free media for routine culture work, 298  
 — Serum veal agar. A dependable substitute for ascitic or blood agar, 295  
 FISCHER, MARTIN H., and HOOKER, MARIAN O. Note on the colloid chemistry of Fehling's sugar test, 368  
 — On the colloid-chemical mimicry of certain enzymatic reactions, 373  
 FLEISHER, MOYER S., and IVES, GEORGE. An antigen for use in complement fixation in tuberculosis, 302  
 FORBES, ROY P., and SNYDER, HELEN A. Study of the leucocytes in an epidemic of influenza, 758  
 FORCE, JOHN NIXSON, and STEVENS, IDA MAY. The responsibility of the vaccinator in overcoming the rational objections to smallpox vaccination, 220

### G

- GAY, FREDERICK P. Recent aspects of streptococcus infection, 721

- GRAVES, STUART. Infectious meningitis—A study of 27 cases in 586 autopsies, 32
- GUILD, STACY R. War deafness and its prevention—A report of tests upon eight preventives, 226, 338
- GUTHRIE, C. C. A tentative explanation of the mechanism of hemolysis associated with loss of water, and the bearing of the phenomenon on certain biologic problems, 87

## H

- HAYS, GEORGE. (*See* Downs and Hays), 553
- HASKELL, CHARLES C., and COURTNEY, R. H. The value of calcium sulphide in the treatment of poisoning by mercuric chloride, 110
- HATCHER, R. A., and SOLLMANN, (*See* Sollmann and Hatcher), 316
- HELLER, EDWARD P. (*See* Reimann and Heller), 238
- HIGGINS, JOHN A. An improved method for anesthetizing animals, 378
- HOLMAN, W. L., AVERY, OSWALD T., KINSSELLA, R. A., and BROWN, J. HOWARD. Recommendations of the committee on a standard routine method for the isolation and identification of hemolytic streptococci from throats, sputa, and pathologic exudates, 618
- HOOKE, MARIAN O. (*See* Fischer and Hooker), 368, 373
- HULTON-FRANKEL, FLORENCE. Tables for use in blood analysis, 548

## I

- ISAACSON, VICTOR I. A rapid colorimetric method for estimating glucose in urine, 289
- IVES, GEORGE. (*See* Fleisher and Ives), 302

## J

- JACKSON, D. E. A method for making graphic records of the movements of certain internal organs, 63
- and PELZ, MORT D. A contribution to the physiology and pharmacology of chelonian lungs, 344
- and — (*See* Pelz and Jackson), 387
- JACOBS, PHILIP P. Military antituberculosis program perfected, 314
- JAMIESON, WALTER A. On the relation of peptone to biological reactions, 614

## K

- KELLERT, ELLIS. An outline for the combined teaching of pathology and bacteriology in small medical colleges, 416
- KELLY, J. B. (*See* Pryer and Kelly), 269
- KIELY, CHARLES E. A case of symmetrical peripheral gangrene, 352
- KIMBALL, O. P. (*See* Marine and Kimball), 41
- KINSSELLA, R. A. (*See* Holman, Avery, Kinsella, and Brown), 618
- KOLMER, JOHN A. The demand for and training of laboratory technicians, 493

## L

- LANDENBERGER, L. L. (*See* Morse and Landenberger), 557
- LANGDON, FLETCHER. The mastic test for the diagnosis of cerebrospinal syphilis, 376
- LEVINSON, A. Measurement of the spinal puncture needle, 127
- LEVY, M. D. (*See* McNeil and Levy), 18
- LINTZ, WILLIAM. Researches in rheumatism, 509
- LUCKHARDT, A. B. Note on a uniformly satisfactory method of collecting urine separately from each ureter in acute experimental work (dogs), 558
- LUDEX, GEORGINE. Studies on Cholesterol—III. Influence of bile derivatives in Bloor's cholesterol determination, 93
- Studies on Cholesterol—IV. Experiments concerning the relation of the diet, the blood cholesterol, and the lymphoid defense, 141

## M

- MACLEOD, J. J. R. Acapnia and shock (*E*), 442
- Functional heart tests (*E*), 436
- Investigations on shock at the front (*E*), 503
- Simplified gas analysis, 622
- MANLEY, O. T. (*See* Marine and Manley), 48
- MANWARING, WILFRED H. Introductory exercises in experimental pathology, 761
- MARCHAND, JOHN L. Tolerance and immunity, 571
- MARINE, DAVID, and KIMBALL, O. P. The prevention of simple goiter in man, 41



- MARINE, DAVID, and MANLEY, O. T. Transplantation of the thymus in rabbits—Relation of the thymus to sexual maturity, 48
- MCGUIGAN, HUGH. Sugar metabolism and diabetes, 319
- McMURTRIE, DOUGLAS C. Duty of the employer in the reconstruction of the crippled soldier (*E*), 630
- MCNEIL, H. L., and LEVY, M. D., Observations on the blood and urine ammonia in acidosis, 18
- MOORE, JOSIAH J. Chronic tonsil infections, 283
- MOORE, WILLIAM. Methods of control of the clothes louse [*pediculus humanus (vestimenti)*], 261
- MORSE, WITHROW, and LANDENBERGER, L. L. A simple mounting for the carbon dioxide apparatus of Van Slyke, 557

## N

- NOBLE, ARLYLE. (*See* Ferry and Noble), 295, 298

## O

- O'SHANSKY, A. L. (*See* Bartlett and O'Shansky), 118

## P

- PALMER, GEORGE T. (*See* Vaughan and Palmer), 635
- PEARCE, R. G. A clinical method for determining the respiratory exchange in man, 420
- Further researches on the physiology of the adrenals (*E*), 441
- Hoover's diagnostic signs elicited from the movements of ribs, 497
- The nervous mechanism in thyroid secretion (*E*), 380
- PELZ, MORT D., and JACKSON, D. E. An investigation of certain phenomena of allergy with special reference to the respiratory and circulatory systems in relation to the cause of death, 387
- and — (*See* Jackson and Pelz), 344
- PETTIBONE, DOROTHY FOSTER. The factors of coagulation in the blood in certain pathologic conditions, 275
- PRYER, R. W., and KELLY, J. B. The etiology of scarlet fever.—1. A study of organisms found in the blood of scarlet fever patients, 269
- and SEWELL, GEORGE. The etiology of scarlet fever, 525

## R

- REIMANN, STANLEY P., and HELLER, EDWARD P. A compact box for the collection and transportation of blood for hemoglobin estimations and cell counts, 238
- RICHEY, DEWAYNE G. Massive infarction of spleen with report of a case, 519
- RIST, E. The bearing of antityphoid vaccination on the diagnostic value of the agglutination test in typhoid and paratyphoid fever, 1
- ROGOFF, J. M. On the liberation of epinephrin from the adrenal glands—With discussion of some of the methods employed in its investigation, 209
- ROWE, L. W. The intravenous use of red mercuric iodide, 412

## S

- SEELMAN, J. J. Simple method of measuring antisheep amboceptor content of human serum and correcting for it in Wassermann tests, 626
- SEWELL, GEORGE. (*See* Pryer and Sewell), 525
- SNYDER, HELEN A. (*See* Forbes and Snyder), 758
- SOLLMANN, TORALD, and HATCHER, R. A. Reporting of accidents from local anesthetics (*E*), 316
- STARK, J. R. The germicidal value of the common gynecologic douching agents, 199
- STEVENS, IDA MAY. (*See* Force and Stevens), 220
- STIEGLITZ, JULIUS. Procaine and Novocaine identical (*E*), 569

## T

- THOMPSON, LEONARD R. A device for accurate pipetting, 130

## V

- VAN SAUN, ANNA L. The effect of the natural antisheep hemolysin content of human serum on complement-fixation tests, 60
- The Wassermann reaction with large amounts of patient's serum, 61
- VAUGHAN, VICTOR C. An explosive epidemic of influenzal disease at Fort Oglethorpe (*E*), 560
- Disordered action of the heart among soldiers (*E*), 134
- Epidemic bronchitis at Fort Oglethorpe, Georgia, (*E*), 567

- VAUGHAN, VICTOR C. Goldberger's studies of pellagra (*E*), 306
- Measles and pneumonia in our camps (*E*), 248
  - Our former teachers in Germany (*E*), 205
  - The control of communicable disease among our soldiers (*E*), 311
  - The use of atropine as a diagnostic agent in typhoid infections (*E*), 258
  - The use of poisonous gases in the present war (*E*), 70
  - and PALMER, GEORGE T. Communicable diseases in the national guard and national army of the United States during the six months from September 29, 1917, to March 29, 1918, 635
- VAUGHAN, WARREN T. Adaptations of renal functions tests for general use, 531

## W

- WALKER, O. J. and BRUCE, W. J. A substitute for white mice in pneumococcus grouping, 434
- WARD, HERBERT C., and BEAVER, DONALD C. Bacteriologic findings in ozena—second report, 348
- WARFIELD, LOUIS M. The etiology of arteriosclerosis, 115
- WARTHIN, ALDRED SCOTT, and WELLER, CARL VERNON. The pathology of the skin lesions produced by mustard gas (dichlorethylsulphide), 447
- WEBB, GERALD B. Cigarette smokers and pulmonary tuberculosis (*E*), 769
- Colonel George E. Bushnell, Medical Corps (*E*), 440
  - Culture methods for the isolation of *B. tuberculosis* (*E*), 207
  - Immunity to tuberculosis (*E*), 383
  - Lymphocyte elements and tuberculosis (*E*), 385
  - Pulmonary tuberculosis (*E*), 767
  - Tuberculosis and the army (*E*), 137
- WEIR, J. W. An inexpensive colorimeter, 132
- Modifications of the Soxhlet extractor, 204
- WELLER, CARL VERNON. (*See* Warthin and Weller), 447
- WOHL, M. G. A simple method of obtaining blood serum, 68
- WOOLLEY, PAUL G. A case of congenital cystic kidney in which a tuberculous process was superimposed, 55
- Convulsions (*E*), 719
  - Epidemic meningitis and its treatment (*E*), 564
  - Lymphocytes and cancer (*E*), 139
  - Meningitis at Camp Greene, 409
  - Pneumonia and meningitis, 602
  - Substitutes for blood in transfusion (*E*), 629
  - Three cases of parietal aortic thrombosis, 539
  - Three cases which illustrate the consequences of coronary lesions, 192

## SUBJECT INDEX

### A

Acapnia and shock, 442  
 Accidents from local anesthetics, reporting, 316  
 Acidosis, observations on blood and urine ammonia in, 18  
 Actinomycosis of the tonsil, 286  
 Activity of double tungstates of alkaloids, 187  
 Adrenal glands, liberation of epinephrin from, 209  
 Adrenals, further researches on the physiology of the, 441  
 Aerobic growths, 511, 512  
 Agar, serum veal, 295  
 Agglutination test in typhoid and paratyphoid fever, 1  
 Alkaloids, tungstates of, 179  
 Allergy, phenomena of, with reference to respiratory and circulatory system in relation to the cause of death, 387  
 Ambard test, 536  
 American peptones, 614  
 Ammonia, blood and urine, observation on, in acidosis, 18  
 Anaerobic cultural characteristics, 515  
 Analysis of peptone, 76, 82  
 Anaphylactic response produced after injection of antigen, 388, 389  
 Anaphylactic shock, 387  
 Anaphylatoxin, 593  
 Anesthetics, local, reporting accidents from, 316  
 Anesthetizing animals, improved method for, 378  
 Animal experiments with mustard gas, 468  
 Antibodies in gonococcal arthritis after the intravenous injection of specific and nonspecific protein, 114  
 Antigen, correlation of fixation without, 404  
   for use in complement fixation in tuberculosis, 302  
 Antisheep amboceptor content of human serum, simple method of measuring and correcting for, in Wassermann test, 626  
 Antituberculosis, military, program perfected, 314  
 Antityphoid vaccination, the bearing of, on diagnostic value of the agglutination test in typhoid and paratyphoid fever, 1  
 Aortic thrombosis, parietal, 539  
 Apparatus for testing devices for prevention of deafness, 338  
   for the teaching laboratory, 553

### Apparatus—Cont'd

  used in determining the respiratory exchange in man, 420  
 Army and civil life, comparative mortality in, 636  
 Army and tuberculosis, 137  
 Army camps:  
   army and tuberculosis, 137  
   association with civilian community as cause of disease in army camps, 688  
   bronchitis, epidemic at Fort Oglethorpe, 567  
   Camp Greene, diseases in, 610  
   meningitis at, 409  
   climatic influence on disease incidence in army camps, 695  
   communicable diseases among our soldiers, control of, 311  
   contact with carrier cases, a cause of disease in army camps, 685  
   diphtheria in army camps, 674  
   disordered action of the heart among soldiers, 134  
   epidemic bronchitis at Fort Oglethorpe, 567  
   epidemic bronchitis in army camps, 675  
   epidemic disease in National Guard and National Army camps, 649  
   epidemic of influenzal disease at Fort Oglethorpe, 561  
   exposure to severe weather as cause of disease in army camps, 681  
   fatigue, as cause of disease in army camps, 684  
   Fort Oglethorpe, epidemic bronchitis at, 567  
   Fort Oglethorpe, influenzal disease at, 560  
   heart, disordered action of the, among soldiers, 134  
   hookworm infection lessens resistance of men in southern camps, 256  
   inadequate hospital care of patients, as cause of disease in army camps, 690  
   inadequate housing as cause of disease in army camps, 684  
   influenzal disease at Fort Oglethorpe, an explosive epidemic of, 560  
   influenza in army camps, 676  
   importation of mildly sick men from other camps as cause of disease in army camps, 687  
   insufficient clothing as cause of disease in army camps, 683  
   measles and pneumonia in our camps, 248

- Army Camps—Cont'd  
 measles in, 607, 662  
 meningitis at Camp Greene, 409  
 meningitis in, 608, 666  
 military antituberculosis program perfected, 314  
 overcrowded quarters as cause of disease in army camps, 689  
 paratyphoid and typhoid in army camps, 672  
 pneumonia and measles in our camps, 249  
 pneumonia and other respiratory diseases, protection against, in army camps, 660  
 pneumonia in, 603, 649  
 pneumonia, susceptibility of men in different camps and experiences of the Civil War in this connection, 655  
 racial influence on cause of disease in army camps, 692  
 scarlet fever in army camps, 668  
 tuberculosis in army camps, 674  
 typhoid and paratyphoid in army camps, 672  
 unsanitary conditions as cause of disease in army camps, 691  
 volunteer medical service corps, 381  
 Army, causes of death in the, 645  
 death rates in, 664  
 sickness in, 648  
 Arteriosclerosis, etiology of, 115  
 Ascitic agar, serum veal agar, a substitute for, 295  
 Association with civilian community as cause of diseases in army camps, 688  
 Atrophic rhinitis fetid, 349  
 Atropine as a diagnostic agent in typhoid infection, 258  
 Automatic key, 553

## B

- Bacillus diphtheroid in ozena, 349, 350  
 mucosus capsulatus in ozena, 349, 350  
 tuberculosis, culture methods for isolation of, 207  
 Bacteria diphtheriæ, historical resume of, 358  
 experimentation with, method of, 359  
 Bacterial utilization of sample peptones, 84  
 Bacteriologic findings in ozena, 348  
 investigations in rheumatism, 511  
 Bacteriology and pathology, combined teaching of, in small medical colleges, 416  
 Besredka's antigen in complement-fixation test, 50  
 Bile derivatives from icteric blood in glacial acetic acid, 105

- Bile—Cont'd  
 in Bloor's cholesterol determination, influence of, 93  
 green in glacial acetic acid, 104  
 Biologic reactions relation of peptone to, 614  
 Bleeding, method of, 270  
 Blood agar plates for use in standard isolation of hemolytic streptococci, 618, 620  
 serum veal agar a substitute for, 295  
 ammonia content in normal individuals, 19  
 in acidosis, observations on, 18  
 analysis, tables for, 548  
 cholesterol, lymphoid defense, and diet, experiments concerning the relation of, 141  
 coagulation of, in certain pathologic conditions, 274  
 difference between normal and diabetic, 329  
 gases, pump for removing and analyzing of, 623  
 in transfusion, substitutes for, 629  
 pressure in shock at the front, 505  
 taking of, in renal tests, 532  
 serum, a simple method of obtaining, 68  
 stagnation in shock, 505  
 sugar, conditions reducing, 334  
 Bouillon culture for use in standard isolation of hemolytic streptococci, 619, 620  
 Box, a compact, for the collection and transportation of blood for hemoglobin estimations and cell counts, 238  
 Bronchitis, epidemic, at Fort Oglethorpe, 567  
 Bronchoconstriction in a well-sensitized dog upon injection of horse serum, 391, 393  
 Bronchopneumonia, 604  
 predominance of, in army camps, 604  
 streptococci in, 736  
 Bushnell, Colonel George E., 441

## C

- Calcium sulphide, value of, in the treatment of poisoning by mercuric chloride, 110  
 Caloric value calculated from the gas exchange, 430  
 Calculation of the percentile composition of the air of the respiratory quotient, 427  
 Camp Greene, diseases in, 610  
 meningitis at, 409  
 Cancer and lymphocytes, 139



- Carbohydrates, influence of excess of, on cholesterol content and cytology of the blood, 152
- Carbon dioxide apparatus of Van Slyke, a simple mounting for, 557
- Cardiac failure, 21  
hypertrophy and dilatation, 195
- Carriers of infectious diseases, 602
- Cerebrospinal syphilis, mastic test for the diagnosis of, 376
- Chelonian lungs, physiology and pharmacology of, 344
- Chemical composition and biologic availability of peptone, an investigation of, 75
- Chemical-fastness of gonococci, 491
- Chemotherapy in streptococcus infections, 751
- Chlorpicrin as fumigant for destruction of clothes louse, 266
- Cholesterol, blood, influence of digestion on, 145  
content of food, 144  
in glacial acetic acid, 104  
solutions color reactions in, 98  
standard of the blood, 143  
studies in, 93  
studies on, 141
- Chronic diseases of the liver, 21  
tonsil infections, 283
- Cigarette smokers and pulmonary tuberculosis, 769
- Climatic influence on disease incidence in army camps, 695
- Clothes louse, methods of control of, 261
- Coagulation, factors of, in the blood in certain pathologic conditions, 275  
of blood in epilepsy, 277  
of blood in hemophilia, 277  
of blood in hemorrhagic purpura, 278  
of blood in jaundice, 278  
of blood in myelogenous leukemia, 279
- Colloid-chemical mimicry of certain enzymatic reactions, 373
- Colloid chemistry of Fehling's sugar test, note on, 368
- Colorimeter, inexpensive, 132  
method for estimating glucose in urine, 289  
solutions used in, 289  
standards in, 289  
technic of, 290
- Communicable diseases among our soldiers, control of, 311  
in the National Guard and National Army of the United States during six months from September 28, 1917, to March 29, 1918, 635
- Complement fixation, immunity with reference to, 397  
in tuberculosis, antigen for use in, 302
- Complement fixation—Cont'd  
test, effect of natural antisheep hemolysin content of human serum on, 59  
in tuberculosis, with Besredka's antigen, 50  
without any antigen, 404
- Congenital cystic kidney in which a tuberculous process was superimposed, 55
- Contact with carrier cases as cause of disease in army camps, 685
- Container for slides, 245
- Convulsions, 719
- Coronary lesions, three cases illustrating consequences of, 192
- Cotton as ear protector, 227  
plugs as ear protectors, results of tests on, 342
- Creatinine, table for use in blood analysis, 550
- Crippled soldier, duty of the employer in the reconstruction of the, 630
- Culture methods for isolation of *B. tuberculosis*, 207
- Cultures, method of making, from scarlet fever cases, 270
- Cystic kidney, congenital, in which a tuberculous process was superimposed, 55

## D

- Deafness, war, and its prevention, 338
- Death in the army, causes of, 645
- Death rates in National Guard and National Army, 604
- Detoxication, method of, 574
- Device for accurate pipetting, 130
- Device for the determination of time of muscular contraction and relaxation, 553
- Devices for the prevention of war deafness, 226  
for prevention of war deafness, tests upon, 340
- Diabetes and sugar metabolism, 319
- Diagnosis of tuberculosis, early, by use of x-rayed guinea pig, 175
- Diagnostic signs, Hoover's, elicited from the movements of the ribs, 497
- Dialyzing apparatus not requiring anticoagulants, 323
- Dichlorethylsulphide, action of, on human skin, 456  
pathology of skin lesions produced by, 417
- Diet, blood cholesterol and the lymphoid defense, experiments concerning the relation of, 141  
effect of, on the cholesterol content and the cytology of the blood, 146

- Diphtheria in army camps, 674
  - toxin, studies on, 358
- Digestion, influence of, on the blood cholesterol, 145
- Diphtheroid organism found in scarlet fever cases, 270
  - morphology of, 271
  - organisms, cultural characteristics of, 272
- Diplococcus, gram-positive, microorganism isolated in rheumatism, 511
- Disease in the army, analysis of causes of, 679
- Disordered action of the heart among soldiers, 134
- Douching agents, gynecologic, germicidal value of, 199
- Douglas bag, 423
- Drug, influence of, in diminishing content of epinephrin in adrenals, 216

## E

- Egg albumen, response in animal sensitized to, 391, 392, 394
- Elective affinity of streptococci, 741
- Elliott "Perfect Ear Protector," 227
  - Swimmer, results of tests in, 341
- Emetine, double tungstates of, 189
- Employer, duty of, in reconstruction of the crippled soldier, 630
- Energy function of adrenal glands, 217
- Enzymatic reactions, colloid-chemical mimicry of, 373
- Epidemic bronchitis at Fort Oglethorpe, 567
  - in army camps, 675
  - disease in National Guard and National Army camps, 649
  - meningitis and its treatment, 564
  - of influenzal disease at Fort Oglethorpe, 561
  - sore throat, streptococci in, 732
- Epilepsy, coagulation of the blood in, 277
- Epinephrin in blood, a means of detecting, 210
  - liberation of, from the adrenal glands, 209
- Etiology of arteriosclerosis, 115
- Experimental pathology, introductory exercises in, 761
- Exposure to severe weather as cause of disease in army camps, 681
- Eye lesions produced by mustard gas, 449

## F

- Fatigue as cause of disease in army camps, 684
- Fehling's sugar test, colloidal chemistry of, 368
- Food, cholesterol content of, 144
- Fort Oglethorpe, epidemic bronchitis at, 567
  - influenzal disease at, 560

- Friedländer group, 348
- Fumigation for destruction of clothes louse, 266
- Functional heart tests, 436

## G

- Gangrene, symmetrical peripheral, 352
  - autopsy findings in, 353
- Gas analysis, simplified, 622
- Germany, our former teachers in, 205
- Germicidal value of common gynecologic douching agents, 199
- Glucose, rapid colorimetric method of estimating in urine, 289
- Glycogen, discovery of, 321
- Glycuronates in the urine, a simple test for, 300
- Goiter, age in relation to, 45
  - prevalent in certain localities, 46
  - prophylactic treatment of, 42
  - sex in relation to, 42
  - simple, prevention of, in man, 41
- Gonococcal arthritis, antibodies in, after the intravenous injection of specific and nonspecific protein, 11
- Gonococci, chemical-fastness of, 491
  - silver-fastness of, 487
- Gonococcal action of protein silver solution in vitro, 487
- Goldberger's studies of pellagra, 306
- Graphic records of movements of certain internal organs, method for making, 63
- Guns used in tests on devices for prevention of deafness, 339
- Gynecologic douching agents, germicidal value of, 199

## H

- Hanging-drop arrangement, 246
- Heart, disordered action of the, among soldiers, 134
  - tests, functional, 436
- Hemolysin, 722
- Hemolysis by freezing and thawing, 88
  - by hypertonic solutions, 88
  - mechanism of, associated with loss of water, and the bearing of the phenomenon on certain biologic problems, 87
- Hemolytic index, 119
  - properties in pregnant urine, 405
  - streptococci in diseases involving the throat and respiratory tract, 731
- Hemophilia, coagulation of the blood in, 275
- Hemorrhagic purpura, coagulation of blood in, 278
- H-ion concentration and toxicogenicity changes during growth of *Bact. diphtheriae* in bouillon, 364

Haldane gas analysis apparatus, 424  
 Holman's classification of streptococci, 727  
 Hookworm infection lessens resistance of men in southern camps, 256  
 Hoover's diagnostic signs elicited from the movements of the ribs, 497  
 Hydrogen-ion concentration and toxicogenicity determinations with Bact. diphtheriae, 358

## I

Immunity and tolerance, 572  
   in streptococcus infections, 744  
   in tuberculosis, 383  
   mechanism of, 573  
   reactions, classification of the streptococci on the basis of, 728  
   studies on, with reference to complement fixation, 396  
   to scarlet fever, skin test which may indicate, 525  
 Importation of mildly sick men from other camps as cause of diseases in army camps, 687  
 Impregnation of underwear to control clothes louse, 265  
 Inadequate hospital care of patients as cause of disease in army camps, 690  
 Inadequate housing as cause of disease in army camps, 684  
 Infarction of spleen, massive, 519  
   of papillary muscle, 195  
 Infectious agents causing meningitis, 36  
   meningitis, 32  
 Influenza in army camps, 676  
   leucocytes in an epidemic of, 758  
 Influenzal disease at Fort Oglethorpe, an explosive epidemic of, 560  
 Insufficient clothing as cause of disease in army camps, 683  
 Internal organs, method for making graphic records of movements of, 63  
 Interstitial tonsillitis, chronic, 284  
 Intestine, action of eserine on, 67  
 Intravenous use of red mercuric iodide, 412

## J

Jaundice, coagulation of blood in, 278

## L

Laboratory efficiency, aids to, 241  
   technicians, demand for, and training of, 493  
 Lacrimal tonsillitis, chronic, 284  
 Lesions of the respiratory tract produced by mustard gas, 449  
 Leucocytes in an epidemic of influenza, study of the, 758

Lipoids concerned in growth, 480  
 Louse, clothes, methods of control of, 261  
   powders, 263  
     composition of, 264  
 Lung, contraction of, following vagus stimulation, 346, 347  
   dilatation of, produced by sympathetic stimulation, 346, 347  
 Lymphocyte elements and tuberculosis, 385  
 Lymphocytes and cancer, 139  
 Lymphocytosis in syphilis, 398  
   in tuberculosis, 398  
 Lymphoid defense, diet, and blood cholesterol, experiments concerning the relation of, 141  
 Lysin curve after intravenous injection of killed gonococci, 13

## M

Mallock-Armstrong Ear Defender, results of tests on, 341  
   Ear Protector, 227  
 Massive infarction of spleen, 519  
 Mastic test for diagnosis of cerebrospinal syphilis, 376  
 McLean's index of urea excretion and blood urea nitrogen content, 536  
 Measles and pneumonia in our camps, 248  
   in army camps, 662  
   streptococci in, 735  
 Measurement of the spinal puncture needle, 127  
 Medical service corps, 381  
 Meningitis, age, sex, and race in, 37  
   and pneumonia, 602  
   at Camp Greene, 409  
   epidemic, and its treatment, 564  
   in army camps, 608, 666  
   infectious, 32  
     agents and routes of entrance, 36  
     signs and symptoms of, 38  
     transmission of, 602  
 Mercuric chloride poisoning, calcium sulphide in treatment of, 110  
 Metabolism, definition of, 320  
 Microscopic appearances of skin lesions after application of mustard gas, 461  
   pathology of animal lesions produced by mustard gas, 471  
   shelf, 244  
 Middle ear parts, observations on the, in tests on ear protecting devices, 231  
 Military antituberculosis program perfected, 314  
 Mortality in army and civil life, 636  
 Mosenthal's special two-hour renal test, 533  
 Mounting, simple for carbon dioxide apparatus of Van Slyke, 557  
 Muscular contraction and relaxation, device for determining time of, 553

- Mustard gas, experimental production of lesions with, 451  
 eye lesions produced by, 449  
 pathology of skin lesions produced by, 447  
 prevention of skin lesion after application of, 468  
 Mutation in streptococci, 729  
 Myelogenous leucemia, coagulation of blood in, 279  
 Myocardial fatty degeneration, 195

## N

- Naphthalene, evaporation of, from jars closed with different kind of cloth, 262  
 National Army, communicable diseases in, 635  
 Guard, communicable diseases in, 635  
 death rates in, 664  
 Natural susceptibility to disease in army camps, 692  
 Needle, measurement of the spinal puncture, 127  
 Nephritis, cases of, 28  
 Nervous mechanism in thyroid secretion, 380  
 Neutrophils in the blood in various conditions, 163-171  
 Nonprotein nitrogen, table for use in blood analysis, 549  
 Nonspecific protein medication, 574  
 Novocaine and procaine identical, 569

## O

- Oils, use of, in control of clothes louse, 265  
 Opsonin curve after intravenous injection of killed gonococci, 13  
 Ophthalmoscopic examination, in renal tests, 533  
 Organisms found in blood of scarlet fever patients, 269  
 Overcrowded quarters, as cause of disease in army camps, 689  
 Ozena, bacteriologic findings in, 348

## P

- Paradichlorobenzene, evaporation of, from jars closed with different kinds of cloth, 262  
 Paratyphoid and typhoid in army camps, 672  
 in nonvaccinated individuals, 8  
 in previously vaccinated cases, 6  
 Parenteral introduction of protein, 575  
 Parietal aortic thrombosis, 539  
 Passive immunization by antisera in streptococcus infections, 747

- Pathogenicity of polymorphic organism for animals, 274  
 Pathology and bacteriology, combined teaching of, in small medical colleges, 416  
 experimental introductory exercises in, 761  
 Pediculus humanus, methods of control of, 261  
 vestimenti, methods of control of, 261  
 Pellagra, Goldberger's studies of, 306  
 Peptone, an investigation of the chemical composition and biologic availability of, 75  
 analysis of, 76, 82  
 free media for routine culture work, 299  
 hypoglycemia, 335  
 properties of samples, 79  
 reactions of samples of, 80  
 relation of, to biologic reactions, 614  
 selection of, for production of diphtheria toxin, 614  
 Peptones, American, 614  
 Perez group, 348  
 Peripheral gangrene, symmetrical, 352  
 Peritonitis, chronic, 285  
 Pharmacology of chelonian lungs, 344  
 Phenolsulphonephthalein test, 535  
 Physiology of chelonian lungs, 344  
 of the adrenals, further researches on the, 441  
 Pigment solutions, color reactions in, 100  
 Pipette rack, 243  
 Pipetting, device for accurate, 130  
 Pneumococcus grouping, a substitute for white mice in, 434  
 Pneumonia a menace in the army camps, 608  
 and measles in our camps, 249  
 and meningitis, 602  
 and other respiratory diseases, protection against in army camps, 660  
 complicated by measles, 606  
 in the army camp, 649  
 susceptibility of men in different camps and experiences of the Civil War in this connection, 655  
 types of, 603  
 virulence of infecting organisms causing, 653  
 Poisonous gases, use of, in present war, 70  
 Poliomyelitis, anterior, streptococci in, 743  
 Polychromatic toluidin-blue stain, a highly differentiating, 432  
 Polymorphic organism in scarlet fever, 272  
 morphologic and staining characteristics, 273  
 Potassium silicotungstate, preparation of, 180  
 Powders, louse, 263  
 composition of, 264



Prevention of simple goiter in man, 40  
 Preventives of war deafness, 226  
 Procaine and novocaine identical, 569  
 Protein, action produced by injection of,  
   into sensitized dog, 388, 390  
   antibodies in gonococcal arthritis after  
   the intravenous injection of spe-  
   cific and nonspecific, 11  
   cleavage, 578  
   medication, nonspecific, 575  
   poison in immunology, 571  
   poison not specific, 600  
   sensitization and bacterial immunity iden-  
   tical, 600  
   theory of, 573  
   silver solution, gonococidal action of,  
   487  
   toxicity of, 575  
 Prothrombin test, technic of, 280  
 Pulmonary tuberculosis, 767  
   and cigarette smokers, 769  
 Pump for removing and analyzing the blood  
   gases, 622

## Q

Quinine silicotungstate, 190

## R

Racial influence on cause of disease in  
   army camps, 692  
 Reconstruction of the crippled soldier, duty  
   of the employer in, 630  
 Red mercuric iodide, intravenous use of,  
   412  
   toxicity of, 413  
 Renal efficiency, outline for a complete ex-  
   amination of, 532  
   function tests for general use, 531  
 Respiratory diseases in army camps, sum-  
   mary of causes of, 699  
   exchange in man, a clinical method for  
   determining, 420  
 Responsibility of the vaccinator in over-  
   coming the rational objections to  
   smallpox vaccination, 220  
 Rheumatic fever group, streptococcus viri-  
   dans in connection with, 740  
 Rheumatism, bacteriologic investigation in,  
   511  
   caused by a microorganism, 510  
   researches in, 509  
 Root-filled teeth, an experimental study of,  
   202

## S

Sachets, destruction of louse by means of,  
   261  
 Scarletina, 526  
 Scarletina tests, 528

Scarlet fever, etiology of, 269, 525  
   in army camps, 668  
   large polymorphic organism in, 272  
   streptococci in, 734  
 Scientific ear drum protector "Tommy",  
   226  
   results of tests on, 341  
 Sensitization a specific phenomenon, 573  
 Serum pipettes, 246  
 Serum veal agar a dependable substitute  
   for ascitic or blood agar, 295  
 Sexual maturity, thymus in relation to, 48  
 Shock and acapnia, 442  
   at the front, investigations of, 503  
   influence of, on secretion of epinephrin  
   from adrenals, 217  
   low blood pressure in, 505  
 Sickness in the army, 648  
 Silver-tastness of gonococci, 487  
 Silver solution, effect of age of, 489  
   effect of exposure of, to light, 489  
   effect of heat on, 490  
 Simplified gas analysis, 622  
 Siphon bottles, 242  
 Skin lesions produced by mustard gas  
   (dichlorethylsulphide), pathology  
   of, 447  
   produced by mustard gas, illustrated  
   through the various stages, 454,  
   478  
 Skin test which may indicate immunity to  
   scarlet fever, 525  
 Smallpox, streptococci in, 737  
   vaccination, responsibility of vaccinator  
   in overcoming the rational objec-  
   tions to, 220  
 Sodium iodide in prophylactic treatment of  
   simple goiter, 42  
 Soxhlet extractor, modifications of, 202  
 Spinal puncture needle, measurement of,  
   127  
 Spleen, massive infarction of, 519  
 Staphyloprotein, results of tests with, 582  
 Stock cultures of streptococci, 621  
 Streptococci, classification of, on the basis  
   of fermentation of sugar, 725  
   on the basis of immunity reactions,  
   728  
   on the basis of production of a hem-  
   otoxin (hemolysin), 722  
   elective affinity of, 741  
   hemolytic, from throats, sputa, and path-  
   ologic exudates, recommendations  
   of the committee on a standard  
   routine method for the isolation  
   and identification of, 618  
   in diseases involving the throat and  
   respiratory tract, 731  
   Holman's classification of, 727  
   in anterior poliomyelitis, 743  
   in bronchopneumonia, 736

- Streptococci—Cont'd  
 in epidemic sore throat, 732  
 in measles, 735  
 in scarlet fever, 734  
 in smallpox, 735  
 microorganism of rheumatism resembles, 511  
 mutations in, 729  
 Streptococcus infection, aspects of, 721  
 infections, chemotherapy, 751  
 immunity in, 744  
 passive immunization by antisera in, 747  
 preventing and curing, 746  
 longus, 723  
 mucosus, 723  
 viridans, 723  
   in connection with rheumatic fever group, 740  
 Streptoprotein, results of tests with, 582  
 Strychnine phosphotungstate, 187  
 silicotungstate, 188  
 Substitutes for blood in transfusion, 629  
 Sugar, blood, conditions reducing, 334  
   classification of streptococci on the basis of fermentation of, 725  
   definition of, 320  
   in blood, table for, 551  
   metabolism and diabetes, 319  
 Symmetrical peripheral gangrene, 352  
 Syphilis, cerebrospinal, mastic test for the diagnosis of, 376  
   of the tonsil, 286

## T

- Tables for use in blood analysis, 548  
 Teachers in Germany, our former, 205  
 Teaching, combined, of pathology and bacteriology, 416  
 Technicians, laboratory, demand for, and training of, 493  
 Teeth, root-filled, an experimental study of, 203  
 Test:  
   complement-fixation, 59  
   complement-fixation, in tuberculosis, with Besredka's antigen, 50  
   Moscenthal's special two-hour renal, 533  
   phenolsulphonephthalein, 535  
   renal function, 531  
   therapeutic, 537  
   Wassermann, 61, 118  
 Testing devices for prevention of war deafness, 339  
 Testing ear protecting devices, method of, 227  
 Tethelin, clinical observations on the action of, 480  
 Therapeutic test, 537  
 Throat and respiratory tract, hemolytic streptococci in diseases involving the, 731  
 Thrombosis, parietal aortic, 539

- Thymus, relation of, to sexual maturity, 48  
 transplantation of in rabbits, 48  
 Thyroid examinations for goiter, 43  
 Thyroid secretion, nervous mechanism in, 380  
 Tissot spirometer, 423  
 Tolerance and immunity, 572  
 Tonsil infections, chronic, 283  
 Tonsillitis, chronic interstitial, 248  
   chronic lacunar, 284  
 Toxemias of pregnancy, blood ammonia in, 22  
 Toxic vaccines, 577  
 Toxicity of double tungstates, 181  
   of protein, 575  
   of red mercuric iodide, 413  
 Toxicogenicity determinations with Bact. diphtheriæ, 358  
 Toxin, diphtheriæ, studies on, 358  
 Transfusion, substitutes for blood in, 629  
 Tuberculoprotein, results of tests with, 582  
 Tuberculosis and lymphocyte elements, 385  
   and the army 137  
   antigen for use in complement fixation in, 302  
   immunity in, 383  
   in army camps, 674  
   of the tonsil, 285  
   pulmonary, 767  
   and cigarette smokers, 769  
 Tuberculosis process in congenital cystic kidney, 55  
 Tungstates of alkaloids, 179  
 Tungstates, toxicity of double, 181  
 Typhoid and paratyphoid in army camps, 672  
   in nonvaccinated individuals, 8  
   in previously vaccinated cases, 6  
   infection as a diagnostic agent in, 258

## U

- Unsanitary conditions as cause of disease in army camps, 691  
 Urban life in relation to disease, 693  
 Urea, table for use in blood analysis, 551  
 Uric acid, table for use in blood analysis, 552  
 Urine, ammonia in acidosis, observations on, 19  
   diabetic, 320  
   examination in connection with renal tests, 533  
   glycuronates in, a simple test for, 300  
   method of collecting, separately from each ureter in acute experimental work, 558  
   rapid colorimetric method for estimating glucose in, 289  
   titration of, in pregnancy for hemolytic properties, 405  
   titration of normal, for inhibiting properties, 405

Uterine action after ergamine, 65  
contractions after pituitrin and adrenalin,  
64

## V

Vaccination, antityphoid, 1  
comparison of the three types of suc-  
cessful, 223  
failures to secure successful, not evi-  
dences of immunity, 221  
use of small multiple scarifications, 221  
Vaccinator, responsibility of, in overcom-  
ing the rational objections to small-  
pox vaccination, 220  
Vaccine, typhoid, in establishing immunity,  
576  
Vaccines are protein sensitizers, 600  
toxic, 577  
use of, in preventing and curing strepto-  
coccus infections, 746  
Van Slyke's carbon dioxide apparatus, a  
simple mounting for, 557  
Vegetable diet, effect of, on blood chole-  
sterol, 151  
Ventricular aneurysm, 195  
Ventricular thrombosis, 195  
Virulence of infecting organisms causing  
pneumonia, 653  
Volunteer medical service corps, 381

## W

War deafness and its prevention, 226, 338  
War, use of poisonous gases in, 70  
Wash bottle, 243  
Wassermann, modified, technic based upon  
the rapid fixation of complement  
present in human serum, 118  
reaction with large amounts of patient's  
serum, 61  
test, method of measuring antisheep am-  
boceptor content of human serum  
and correcting for it in, 626  
modified, final positive, reading with  
cholesterinized antigen, 121  
Wax cones as ear protectors, 227  
as ear protectors, results of tests on,  
342  
White mice, a substitute for, in pneumo-  
coccus grouping, 434  
Wilson-Michelson device for prevention of  
deafness, results of tests upon, 342  
for protection of the ear, 227

## X

X-rayed guinea pig, early diagnosis of tu-  
berculosis by the use of, 175  
Xylene, evaporation of, from jars closed  
with different kinds of cloth, 262

## VALUABLE SUGGESTIONS FOR CONTRIBUTORS TO THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE

"The four rules for the preparation of an article will then be: (1) Have something to say; (2) Say it; (3) Stop as soon as you have said it; (4) Give the paper a proper title."<sup>1</sup>

Let your phraseology express one meaning and one only. Be clear.<sup>2</sup>

**Manuscript.**—Manuscripts should be typewritten, with wide margins, and double spaced, on one side of paper 8½ by 11 inches in size. The original copy should be sent to the "Journal" and the carbon copy retained by the author. Number the leaves consecutively, beginning with the title page. Put your name and address on the manuscript.

**Illustrations.**—Illustrations should be clear, preferably pen-and-ink drawings. Of photographs send a good print rather than a negative. Have lettering parallel to the bottom and top margins, and of sufficient size to be clear if cut is to be reduced. Tracings should be in black-and-white; avoid colors. Write your name on back of each picture; number them in one series (Fig. 1, etc.) to the end, and indicate in margin of the manuscript about where each is to be printed. See that the text references and "figures" correspond. Legends for illustrations should be written on a separate sheet.<sup>3</sup>

**Bibliographic References.**—Give only references actually consulted. If an article is known only through an abstract give reference to the abstract in addition to that of the source. References are printed to be of help in further reading; therefore they must be complete, concise, and correct. Follow the style of the "Index Medicus" and "Index-Catalog of the Library of the Surgeon-General's Office." Be conservative in the use of abbreviations.<sup>4</sup>

**Arrangement.**—As authors are quoted in the text give each a number in the order of citation, and number the bibliographic reference with the same number. Arrange the references in a list at the end of the article in the order of the numbers (see below), or arrange items in alphabetical order according to last names of authors, and distinguish between articles by the same author by the use of the date after his name in the text.

**Foot-notes.**—Where an author wishes to use foot-notes at bottom of each page instead of the bibliography at end of article, the foot-notes should be written in the text, but separated from it by horizontal lines above and below, or *better*, place them at bottom of each page. Use figures to indicate these foot-notes, and number consecutively (1, 2, 3, etc.) throughout the article. If in addition to the bibliography mentioned above it is desired to use foot-notes on certain pages, these can be indicated by an asterisk (\*).

**Final Reading.**—Let some one other than the author read the manuscript with these directions in mind.

**Shipment.**—Send manuscript flat, postage paid, to the editor, Dr. Victor C. Vaughan, Surgeon-General's Office, Washington, D. C.

**Proof-reading.**—Read carefully, with special attention to spelling of names and bibliographic data. Make corrections *in the margin* only with lines drawn from the revision to the point of change in the text. Answer queries in the proof by making correction or crossing out the query. Verify your references from the sources, not from your carbon copy.

### References. (Read these.)

<sup>1</sup>Billings, J. S.: Our Medical Literature, Trans. VII Intern. Med. Congress, Lond., 1881, i, 54-70.

<sup>2</sup>Mayer, Emil: Medical Literature and its Preparation, Med. Record, N. Y., 1915, lxxxvii, 1019-1021.

Allbutt, T. C.: Notes on the Composition of Scientific Papers. London, Macmillan, 1904.

McCrae, Thomas: The Use of Words, Jour. A. M. A., Chic., 1915, lxxv, 135-139.

<sup>3</sup>Suggestions to Medical Authors, issued by the A. M. A. Press, Chic., A. M. A., [1914 (?)].

<sup>4</sup>Place, F.: Bibliographic Style in Medical Literature. Med. Record, N. Y., 1913, lxxxiii, 157-160.













R            The Journal of Laboratory  
850        and clinical medicine  
J66        v.3  
v.3  
cop.2

Biological  
& Medical  
Serials

PLEASE DO NOT REMOVE  
CARDS OR SLIPS FROM THIS POCKET

---

UNIVERSITY OF TORONTO LIBRARY

---

